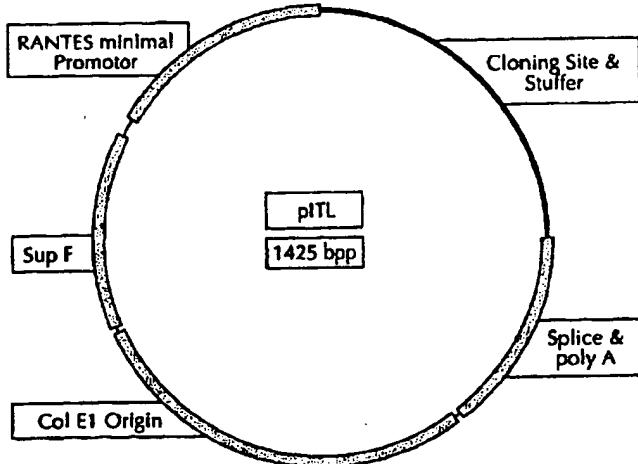




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## (54) Title: A VECTOR FOR POLYNUCLEOTIDE VACCINES



## (57) Abstract

The invention is a "humanized" polynucleotide vector vaccine which uses covalent closed circular plasmid DNA, "naked DNA", to express target antigens. The vector is non-replicating in mammalian cells but is capable of extended stable expression of the target sequences in skeletal muscle and professional antigen presenting cells generating an immune response to the target antigen in immunized individuals. The polynucleotide vector is particularly useful in accommodating monomorphic and polymorphic nucleic acid sequences encoding tumor antigens via PCR technology.

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## A VECTOR FOR POLYNUCLEOTIDE VACCINES

### Field of the Invention

The present invention relates generally to polynucleotide vaccines and a novel vector useful for same. The present invention relates more specifically to a humanized polynucleotide vector vaccine which is useful in generating an immune response to a selected target antigen, in particular, to a tumor antigen.

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### Background of the Invention

Cancer is a leading cause of morbidity and mortality. Conventional therapies such as surgery, radiation treatment and the like have resulted in a modest improvement of survival. There remains a need in the art for alternative treatments. With the advancing knowledge of immunology and tumor interaction with cells of the immune system, the upregulation of the immune system provides an alternative approach to treatment of cancers.

The process of oncogenesis involves multiple steps and predominantly involves intracellular protein products of either oncogenes or tumor suppressor genes (3-7). These intracellular proteins frequently have mutations or alterations leading to dysregulation or altered activity which can result in 'altered self' protein sequences and therefore, potential tumor-specific antigens (8,9). The immune response to any tumor requires recognition of such tumor associated antigens. These more subtle, 'altered self', antigenic differences are likely to be much less immunodominant than truly foreign antigens such as those from infectious disease pathogens or allograft tissues. The recent work defining antigen presentation has revealed that antibodies generally recognize antigens in the form of whole molecules, T cells typically recognize antigens in the form of small peptides combined with MHC molecules. This understanding of antigen presentation has aided the recent identification of a number of tumor-associated antigens which are recognized by T cells and derived from intracellular proteins (9). The characterization of oncogene mutations, dysregulated developmental or oncofetal genes, and mutations of tumor suppressor genes provides multiple potential unique target antigens for anti-tumor immune

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responses.

One form of cancer, breast cancer is the most common cancer affecting women in the United States of America, with 183,400 new cases annually (1). Breast cancer is also the second most frequent single case of female cancer mortality with 46,000 deaths annually (1). Although there has been significant progress over the past several decades in the surgical, radiotherapeutic, and medical treatment of this disease, none of these modalities alone or in combination provide curative therapy for the majority of patients with advanced (stage III or metastatic) breast cancer. Less than 40% of patients with advanced breast carcinoma will be alive and disease free, five years from diagnosis and initial treatment (2). Interestingly, patients with significant inflammatory infiltrates in the primary tumor, i.e. medullary carcinoma, have significantly improved survival (2) despite a higher degree of cellular anaplasia. The recent identification of tumor suppressor gene loss, oncogene mutations and oncogene dysregulation in breast cancers has opened new horizons for the treatment of the extraordinarily common cancer.

Numerous oncogene alterations have been documented in patients with breast adenocarcinoma including p53, HER2/neu (C-erbB-2), Rb, ras, PEM/MUC 1, BRCA1, BRCA2, Int-2, and hst. These particular oncogene alterations yield a panel of potential tumor associated targets for immunomodulatory therapies. Up to 80% of advanced breast adenocarcinomas will have p53 alterations (10-14) with most of the mutations occurring in the so called "hot spot", between codons 130 and 286, (15-20). The C-erbB-2 or HER-2/neu gene product shares homology with the epidermal growth factor receptor (21-25) and is expressed in up to 40% breast carcinomas (26). The tumor suppressor gene Rb has been noted to be altered or absent in 24% to 42% of breast cancer patients (27-29) and this percentage appears to be increased in metastatic lesions over primary tumors. The role of ras alterations appears to be more complex in breast cancer than for most other tumors (30-33). H-ras is the member of this family of proteins which is most frequently altered in breast cancers although, either K ras and/or H ras are overexpressed in excess of 70% of breast cancers. Polymorphic Epithelial Mucin (PEM) or MUC-1 is a tumor-associated antigen which is now known to represent an underglycosylated and dysregulated glycoprotein (34-36) and is diffusely overexpressed on 94% of breast cancers (37). BRCA1 has recently

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been cloned (38) and mutations have been characterized in patients with ovarian and breast cancer (39-43). Int-2 and Hst are oncogene sequences encoding proteins which are members of the fibroblast growth factor (FGF) supergene family (44). Amplification of Int-2 and Hst is significantly correlated with invasive breast carcinoma (45, 46) and estrogen receptor positivity (47). Breast carcinomas have other alterations including overexpression of non-mutated ubiquitous cellular proteins such as c-myc, Bcl-1, Bcl-2, EGFR, PRAD1 (cyclin D1), IGF1R, bFGF, PDGF-B, TGF- $\beta$  and others (48, 49). When the novel or mutated regulatory elements responsible for the overexpression of these individual genes are identified and characterized they could prove to be appropriate target tumor antigens. Clearly, there is a diverse and growing set of potential tumor antigens as a result of the multiple molecular events inherent in the development of breast adenocarcinoma.

Evidence has recently accumulated suggesting a potential for immunotherapeutic approaches in the treatment of breast cancer. Patients with breast cancer can have a cellular response to autologous tumor associated antigens (50). Breast cancer patients can also have antibodies to mutated p53 proteins (51). Elution of antigens from immune complexes present in sera of patients with breast carcinoma reveal multiple oncogenic antigens (52). T-lymphocytes reactive to mutated p53, HER2/neu, and ras peptides have been described (9, 53, 54-58). PEM/MUC-1 has been shown to be recognized by both antibodies and cytotoxic T lymphocyte (CTL) lines (34, 52, 59-61). Some CTL recognize the target antigen in a non-MHC restricted fashion (52, 58-61) however, in a murine model classical HLA restricted CTL recognizing the non-repeating core sequences have been elicited (62, 63). These findings, in addition to the association of improved survival with inflammatory infiltration of primary breast tumors, suggest that patients can mount an immune response to the malignant cells of breast tumors.

Oncogene and tumor suppressor genes can have mutations within confined domains, such as p53 and ras (15-20, 64, 65), while others have mutations which inactivate the protein product and/or can have total loss of the normal alleles, such as RB-1 and BRCA1 (5-7, 66-70). Obviously, allelic deletions provide no protein product but, mutated inactive gene products can be the source of peptides recognized as tumor associated antigens. Significantly, humoral and CTL immune responses to

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polypeptides encompassing the mutated sequences for both p53 and ras have been identified (50-52, 55, 71, 72, 73). These genetic alterations from germline can be highly specific and unique for any one individual tumor, even those with identical phenotypic features, and may yield unique individual tumor antigens (74, 53, 17, 51).

5 Some oncogenes products such as HER2/neu (C-erbB-2), PEM/MUC-1, Int-2, Hst-1, and TRE17 (75-80) do not display polymorphism due to mutations but rather, are overexpressed in malignant tissues and essentially not expressed in normal adult tissues. These proteins and glycoproteins which are preferentially expressed in/on neoplastic mammary cells more closely fit the classical model of tumor antigens.

10 When immune responses have been demonstrated to these preferentially expressed proteins and responses are frequently limited to regions of dissimilarity with the homologous or normal proteins expressed in adult tissues (9, 32, 56, 58, 60). Therefore, regions of dissimilarity, like mutated sequences, are potential antigenic targets for immunomodulation of the anti-tumor immune response.

15 The ability to precisely define and characterize tumor associated antigens has facilitated the design and evaluation of immunomodulatory therapies. Effective immunotherapies have been largely limited to non-Hodgkin's lymphoma and melanoma. In the case of non-Hodgkin's lymphomas, the idiotypic immunoglobulin molecule has been a target of several different strategies (81-84). The use of anti-idiotypic antibodies resulted in some clinical responses but, modulation of the antigen was also seen (85, 86). Use of the intact idiotypic immunoglobulin molecule as a polypeptide vaccine has elicited both cellular and humoral immune responses which correlate with improved clinical courses (87-89). This suggests that even within a single tumor-associated protein a broader immune response, potentially to multiple epitopes within the polypeptide, will result in improved clinical efficacy. Several groups have conducted trials using allogeneic cells or allogeneic cellular preparations in an attempt to address the issue of antigenic heterogeneity within tumors but, with notable exceptions these strategies have not contributed significantly to improved survival in any tumor type (9, 90, 91). Although the evaluation of clinical efficacy rests on improved survival, immunological parameters may be surrogate end points which facilitate the design and evaluation of new strategies. In the case of these "polyvalent" preparations, the ability to adequately evaluate immunologic parameters

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is severely hindered by the lack of defined antigens. To date, there has not been an immunomodulatory trial with multiple, defined, autologous, tumor associated antigens.

Intratumoral heterogeneity dictates that antigen specific immunomodulatory maneuvers be individualized or face severe limitations on the patient population for which they are applicable. Single antigen based immunomodulatory strategies do not account for intratumoral heterogeneity and thus, are at risk for tumor escape from immune surveillance even if effective augmentation of the anti-tumor immune response is accomplished. Based on our current understanding of the biology of neoplasms, including the multitude of cellular processes which must be deranged for transformation and tumorigenesis, each patient's tumor offers multiple potential targets for antigen-specific, immunomodulatory therapies. A more effective and efficacious strategy should take advantage of this multiplicity of potential, highly specific, tumor antigens. However, there is no current immunomodulatory strategy which allows for this individualized 'polyclonal' approach.

We now appreciate that, as with the immune response in allograft rejection or viral infections, an effective anti-tumor immune response must involve a cytolytic cellular response. Evidence is accumulating that the MHC class I pathway of antigen presentation and cytotoxic T lymphocytes (CTL) are important for effective *in vivo* anti-tumor immune responses (62, 89, 92-96). It has been shown that even in vaccine studies using exogenous protein antigen, modest increases in the cytotoxic T lymphocyte compartment have been demonstrated and correlated with freedom from progression (89). Furthermore, the preeminent role of the TH1 phenotype of T helper cells in both humans and animal models supports the critical role of multiple arms of the cellular immune system for an effective anti-tumor immune response (97-101) and provides further evidence for the critical role of CTL anti-tumor immune responses.

The recently described polynucleotide vaccine strategy has been demonstrated to yield humoral and cellular (both helper and cytotoxic T lymphocyte) immune responses to numerous encoded antigens (102, 103, 104-131). This is in contrast to the overwhelming humoral response to exogenous protein or cellular vaccine preparations. This strategy uses covalent closed circular (CCC) plasmid DNA, 'naked DNA', to express the target antigen (117). These plasmids are non-replicating but, are capable of extended stable expression of the target sequences in skeletal muscle

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and potentially in professional antigen presenting cells such as members of the macrophage lineage or dendritic cells (117). The plasmids are non-replicating but, capable of stable mRNA expression within skeletal muscle and reticuloendothelial cells. Thus, antigens encoded by these mRNA sequences are processed as endogenous proteins and presented to the immune system in a manner analogous to presentation of virus encoded protein antigens. It is now recognized that most potential tumor antigens are recognized as peptides derived from intracellular proteins, some of which are dysregulated or mutated oncogene products (103, 132a, 133a, 134a, 104-112, 118, 119). Polynucleotide vaccine strategy provides an avenue for eliciting immune responses to such intracellular oncogene or dysregulated products. Multiple studies have demonstrated the expression of target antigens following intramuscular injection and uptake of 'naked DNA' (132a, 133a, 134a, 103, 1-20). The uptake of DNA is increased if preceded by 24 hours with an intramuscular injection of a myo-toxic agent in the same vicinity (133a, 134a, 1, 7, 10, 15).

The existing vectors use an antibiotic resistance gene as the selection agent. These genes generate a foreign protein, raise the possibility of an anti-vector immune response, and have the theoretical risk of transferring antibiotic resistance to normal host flora. These vectors use the CMV promoter, b actin promoter, or other retroviral LTR elements to drive transcription of the target sequence. These promoter elements have ubiquitous activity but, are large in the case of CMV and b actin, and in the case of the LTR have the theoretical risk of integration and potential oncogenic event. All of the current vectors are designed with a single target antigen in place with little or no flexibility for changing target sequences. The functional size limitation of all plasmid vectors limits the size of insertable elements. By utilizing large promoter elements and the complete ColE1 or MB1 origins of replication the size of the vector is increased and limitations are placed on the size and number of insertable elements. Thus, none of the existing vectors are optimally designed for evaluation of an anti-tumor polynucleotide vaccine strategy.

The majority of the antigens evaluated to date are generally monomorphic and thus, can be readily incorporated into established plasmid vectors. However, putative tumor antigens, i.e., altered self proteins, in any given tumor are not heterogeneous and in some cases distinctly unique. For example, mutations in p53, although limited

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to a contiguous region of approximately 600bp, are very heterogeneous from tumor to tumor (15-20). Other intracellular tumor antigens will likely demonstrate similar heterogeneity in the range of mutations. Therefore, the present invention is a vector which accommodates both monomorphic and polymorphic target antigens via PCR technology. This vector is useful in generating patient-specific, target-specific multiple antigen, anti-target polynucleotide vaccines.

### Summary of the Invention

- 10 An object of the invention is a humanized, polynucleotide vector.
- An object of the invention is a kit comprising a humanized, polynucleotide vector alone, or in combination with at least one nucleic acid sequence encoding one or more target antigens or antigenic epitopes thereof.
- 15 Another object of the invention is a humanized polynucleotide vector vaccine useful in eliciting an immune response against one or more target antigens or antigenic epitope thereof.
- Yet another object of the invention is a pharmaceutical composition comprising the humanized polynucleotide vector vaccine and a pharmaceutically acceptable carrier.
- 20 A further object of the invention is a kit comprising a humanized polynucleotide vector vaccine alone or in combination with an expression enhancing agent.
- Another object of the invention is a sequence acceptance site which accepts cDNA target products from rt-PCR cloning.
- 25 One aspect of the invention is a method of preparing a humanized polynucleotide vector.
- Another aspect of the invention is a method of preparing a humanized polynucleotide vector vaccine.
- 30 Yet another aspect of the invention is a method for expressing at least one target antigen or antigenic epitope thereof in cells comprising introducing a humanized polynucleotide vector into cells under conditions for expression of the target antigen or antigenic epitope thereof.

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Another object of the invention is a host cell expressing at least one target antigen or antigenic epitope thereof provided by a polynucleotide vector.

A further aspect of the invention is a method of stimulating a specific immune response to at least one target antigen or antigenic epitope thereof in a mammal comprising the administration of an effective amount of a polynucleotide vector vaccine into the mammal to elicit the specific immune response.

Another aspect of the invention are target antigen specific monoclonal and polyclonal antibodies elicited by administration of a polynucleotide vector vaccine.

Yet another aspect of the invention are target antigen specific cytotoxic lymphocytes and target antigen specific helper T lymphocytes elicited by administration of a polynucleotide vector vaccine.

These and other objects, features and many of the attendant advantages of the invention will be better understood upon a reading of the detailed description of the invention and drawings.

#### Brief Description of the Drawings

Figure 1 is a genetic map of the polynucleotide vector, pITL.

Figure 2 depicts the insertion site in the polynucleotide vector, pITL, for cDNA encoding target antigen.

Figures 3A through 3C show the amino acid sequence homology between human epidermal growth factor receptor (EGFR) and human Her 2/neu. The line underscores the target sequence of human Her 2/neu. The bold letters indicate the transmembrane domain.

Figures 4A through 4C show the amino acid sequence of human Her 2/neu and Rat Her 2/neu. The lines overlie the target sequence for both human and rat Her 2/neu. The bold letters indicate the transmembrane domain.

Figure 5 shows the restriction enzyme sites of the polynucleotide vector pITL.

#### Detailed Description Of The Invention

The present invention is a "humanized" vector which has the necessary elements to express mRNA for a target antigen. The resultant translated polypeptides are available for processing into presentable antigens to the immune system. The vector accommodates monomorphic and polymorphic nucleic acid sequences of a

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target antigen or antigens. The vector of the present invention is useful for constructing polynucleotide vector vaccines or "naked DNA" vaccines containing a nucleic acid sequence encoding one or more target antigens.

The polynucleotide vaccines vector of the present invention has the following characteristics:

- (1) is selectable for growth and production of a polynucleotide vaccine product,
- (2) is capable of eukaryotic expression of one or more target antigens or antigenic epitopes thereof,
- (3) is functional in selected tissue and selected cells of the inflammatory immune system,
- (4) has minimal extraneous non-human DNA sequences to minimize potential toxicity, and
- (5) is capable of accepting target nucleic acid sequences from a number of different transcripts.

A polynucleotide vaccine of the invention offers multiple advantages over other vaccine strategies for immunomodulation. Polynucleotide vaccine strategies appear to elicit brisk CTL responses in all models in which this aspect of the immune response has been evaluated as seen in Table 1. The primary advantage of the polynucleotide vaccine of the invention is that the target antigen(s) is expressed as an intracellular polypeptide or peptide and, as such, is processed as a self polypeptide or peptide and appropriately presented on antigen presenting cells.

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TABLE 1

ANTIGEN	MODEL SPECIES	IMMUNE RESPONSES				FUNCTIONAL EFFECT
		HUMORAL	PROLIFERATIVE	CTL	TH1/TH2	
Rabies	Mouse	+		+	TH1	Protective
Plasmodium	Mouse	+		+	TH1	Protective
Influenza, Nucleoprotein	Mouse	+		+	TH1	Protective
Influenza, Hemagglutinin	Mouse	+		+	TH1	Protective
	Mouse	+			TH1	Protective
	Chicken	+			TH1	Protective
	Chicken	+			TH1	Protective
Bovine Herpes Virus - 1	Mouse	+		+	TH1	Decreased Shedding
	Bovine	+			TH1	
HBV, Envelope	Mouse	+			TH1	Protective & Ig Class Switch
HBV, Surface A	Rabbit	+			TH1	
HCV, Core Protein	Mouse	+	+		TH1	
HCV, Nucleocapsid	Mouse	+	+		TH1	
HSV, Glycoprotein D2	Guinea Pig	+			TH1	Protective
HIV-1, GP160	Mouse	+		+	TH1	Reduced Syncytia
HIV-1, GP160	Primate	+		+	TH1	Reduced Syncytia
HIV-1, GP120	Primate	+		+	TH1	Reduced Syncytia

TABLE 1, CONT.

SIV	Primate	+	+	Protective Expression	
BBTA GAL	Primate	+			
Luciferase	Primate	+			
Apolipoprotein E	Rat				
CEA	Mouse	+	+	Protective	
p53	Mouse	+	+		
Factor IX	Mouse	+	+		
h Growth Hormone	Mouse				Expression
Dystrophin	Mouse	+			Expression

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Other added advantages of the polynucleotide vector as a vaccine is that preparation of plasmid DNA is much less labor and time intensive than cellular or protein vaccine preparations. Polynucleotide vaccine preparations are readily purified, sterilized, and DNA preparations are known to have a long shelf life when stored as a precipitate. Technical expertise in standard sterile tissue handling procedures, standard molecular techniques, and established PCR technology is all that is required for generation of the polynucleotide vaccine preparation. Administration is by standard medical procedures performed routinely. Safety concerns are minimal as no retroviral or oncogenic viral elements are used in the present invention. Finally, with the vector of the present invention, the only protein to be transcribed and translated is the target antigen thus, unlike the pox virus vectors or existing polynucleotide vaccine vectors there is no acquired immunity to the vector of the present invention.

The polynucleotide vector of the present invention has minimal exogenous, non-human DNA and has been maximally "humanized". A 'humanized' vector is one in which the majority of the functional elements are derived from or synthesized based on a human element or a mammalian homolog of the human element. The intent of humanization is to achieve sustained expression of a target antigen(s) with no or minimal risk of methylation and genetic downregulation which would prevent or inhibit translation of the target antigen(s). Therefore, the vector does not contain sequences which may increase inactivation by methylation or changes in tertiary structure. Thus, the polynucleotide vector selectively elicits immune responses to the target sequence(s) with little or no immune response to the other components of the polynucleotide vector.

The humanized polynucleotide vector comprises a human derived promoter or mammalian homolog thereof which is functional in a mammalian target tissue and mammalian target cells and a sequence acceptance site which accepts cDNA target products from rtPCR cloning.

There are minimal non-human components in the polynucleotide vector. These components of the polynucleotide vector which are non-human derived components are necessary for production of the vector. These non-human components are the origin of replication which allows replication and growth of the vector in bacterial or yeast host cells and a nucleic acid sequence which allows for selection of recombinant

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plasmids in the bacterial or yeast host cells.

In one embodiment, the plasmid comprises an origin for plasmid replication and growth in bacterial cells, a nucleic acid sequence which allows for selection of recombinant plasmids which is operably linked to a human-derived promoter which is functional in a mammalian target tissue and mammalian target cells, a cloning site for insertion of sequences encoding target antigen(s), a stuffer sequence, a human derived 3' splice sequence, and a human derived poly A sequence.

The origin of replication may be non-human and may be derived from several sources including bacteria and yeast and the like. Such origins of replication include, but are not limited to PMB1, pUB110, pBC16, pSAQS01, pX012, pE194, pC194, pS174pSA2100, pSE3, pAM330, pCG1, pCG4, pHM1519, pSa151, pLS103, pTA1060, pBs81/6, pSC101, R1, RK2, RSF1010, ARS1, R6K, Sa, R300B, Rep, RepA, pRO1614, OriT, OriV, OriW, OriC, OriF, OriP, OriT, OriT, CdE1, pEW27, pE194, fd, F1, F, NR1, p15A, a colE1 origin, functional portions thereof, and the like which are available from plasmids deposited with the American Type Culture Collection (ATCC), Rockville, MD. In a preferred embodiment, the origin of replication is a colE1 origin or functional portion thereof. In one embodiment, the origin of replication for use in constructing the polynucleotide vector is a minimal colE1 origin isolated from the vector pBR327 (ATCC) (Oka, A. et al Molec. Gen. Genet. Vol. 172, 151-159, 1979) comprising the sequence:

GGCCGCGTTG CTGGCGTTT TCCATAGGCT CCGCCCCCT GACGAGCATC  
ACAAAAATCG ACGCTCAAGT CAGAGGTGGC GAAACCCGAC AGGACTATAA  
AGATACCAGG CGTTTCCCCC TGGAAGCTCC CTCGTGCCTC CTCCTGTTCC  
GACCCTGCCG CTTACCGGAT ACCTCTCCGC CTTTCTCCCT TCGGGAAAGCG  
TGGCGCTTTC TCAATGCTCA CGCIGTAGGT ATCTCAGTTC GGTGTAGGTC  
GTTCGCTCCA AGCTGGCTG TGTGCACGAA CCCCCCGTIC AGCCCGACCG  
CTGCGCCTTA TCCGGTAACT ATCGTCTTGA GTCCAACCCG GTAAGACACG  
ACTTATCGCC ACTGGCAGCA GCCACTGGTA ACAGGATTAG CAGAGCGAGG  
TATGTAGGCG GTGCTACAGA GTTCTGAAG TGGTGGCCTA ACTACGGCTA  
CAC (SEQ. ID. NO. 1), and analogs thereof.

The minimal colE1 origin is a minimal size yet functional and provides plasmid replication and growth within permissive strains of E. coli. The colE1 is antiparallel as it reads in the polynucleotide vector and comprises the sequence:

GTGTAGCCGT AGTTAGGCCA CCACCTCAAG AACTCTGTAG CACCGCCTAC

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ATACCTCGCT CTGCTAATCC TGTTACCACT GGCTGCTGCC AGTGGCGATA  
AGTCGTGTCT TACCGGGTTG GACTCAAGAC GATAAGTAC GGATAAGGCG  
CAGCGGTCTGG GCTGAACGGG GGGTCGTGC ACACAGCCC GCTTGGAGCG  
AACGACCTAC ACCGAACCTGA GATACCTACA CCGTGACCAT TGAGAAAGCG  
5 CCACGCTTCC CGAAGGGAGA AAGGCGGACA GGTATCCGGT AAGCGGCAGG  
GTCGGAACAG GAGAGCGCAC GAGGGAGCTT CCAGGGGAA ACGCCTGGTA  
TCTTTATAGT CCTGTCGGGT TTCGCCACCT CTGACTTGAG CGTCGATTT  
10 TGTGATGCTC GTCAAGGGGG CGGAGCCTAT GGAAAAACGC CAGCAACCGCG  
GCC (SEQ. ID. NO. 2), and analogs thereof.

In a preferred embodiment, the colE1 origin of replication in the polynucleotide vector comprises the sequence:

15 CTCGGGCCGCGTTGCTGGCGTTTCCATAGGCTCCGCCCTGACGAG  
CATCACAAAAATCGACGCTCAAGTCAGAGGTGGCAAACCCGACAGGAC  
TATAAAGATACCAGGCCTTCCCCCTGGAAGCTCCCTCGTGCCTCTCCT  
GTTCCGACCCCTGCCGCTTACCGATAACCTGTCCGCCTTCTCCCTCGGGA  
AGCGTGGCGCTTCTCAATGCTCACGCTGTAGGTATCTCAGTCGGTGTA  
GGTCGTTCGCTCCAAGCTGGCTGTGCACGAACCCCCGTTAGCCCG  
20 ACCGCTGCCCTATCCGTAACATCGTCTTGAGTCCAACCCGGTAAGA  
CACGACTTATGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGC  
GAGGTATGTAGGCGGTGCTACAGAGTTCTGAAGTGGTGGCCTAACTACG  
GCTACACTAGAAGGACAGTATTGGTATCTCGCTCTGCTGAAGCCAGTT  
ACCTTCGGAAAAAGAGTTGGTAGCTTGTACCGGCAAACAAACCACCGC  
25 TGGTAGCGGTGGTTTTTGCAAGCAGATTACGCGCAGAAAAAA  
AAGGATCT (SEQ. ID. NO. 26)

This fragment of the colE1 replicon was isolated by digesting pBR327 with Bst Y1 and Ava I. After isolation of the fragment by agarose gel electrophoresis the ends were polished to blunt with klenow fragment (large fragment) of DNA polymerase I. Then this fragment was ligated into pBluescript SK at the Sma I site and orientation was determined such that the orientation of the replicon was anti-parallel to the origin of replication of the pBluescript. The SupF fragment was isolated and ligated as described herein, orientation was again determined such that the SupF was read in an anti-parallel direction to the RANTES promoter and the putative target sequence in the completed vector.

A sequence which will provide a mechanism for selection and growth of recombinant plasmids in bacteria or yeast is provided in the vector construct. However, the polynucleotide vectors does not contain foreign antibiotic resistance genes. The sequences may be non-human derived sequences. The polynucleotide vector uses sequences such as suppressor tRNA genes including but not limited to as

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SupE, SupP, SupD, SupU, SupF, SupZ, glyT, glyU, SerP, psu<sup>i</sup>, psu<sup>i</sup>-C34, psu<sup>i</sup>am, psu<sup>i</sup>OC (Eggertson, G. et al, Am. Soc. Microbiology, vol. 52:354-374, 1988), and synthetic supF complementation tRNA gene, derivatives thereof (Brown, El. et al. Methods in Enzymology Vol. 68:109-51, 1979) and the like to provide a mechanism for selection and growth.

The synthetic supF complementation tRNA gene (143) provides the mechanism for selection and growth of recombinant plasmids in a manner analogous to that used in eukaryotic expression cloning using the 'Seed' vector (pCDM8 or derivatives). This selection is dependent upon the presence of the 60kb p3 helper plasmid which contains inactive tetracycline and ampicillin resistance genes due to amber stop codon mutations which are complimented by the supF tRNA. The p3 helper plasmid is derived from pLM-2 as described in Mindich, L. et al. J. Bacteriology vol. 126, pp. 177-182, 1976. The supF tRNA is not functional in eukaryotic cells.

In one embodiment, a Sup F gene sequence for use in the polynucleotide vector comprises:

GAATTCTTTC GGACTTTGAA AAGTGATGGT CGTGGCCGAA GGATTCGAAC  
CTTCGAAGTC GATGACGGCA GATTTAGAGT CTGCTCCCTT TGGCCGCTCG  
GGAACCCCAC CACGGGTAAAT GCTTTACTG GCCTGCTCCC TTATCGGGAA  
GCGGGCGCA TCATATCAAA TGACGGCCG CTGTAAAGTG TTACGTTGAG  
AAAGAATTC (SEQ. ID. NO. 3), and analogs thereof.

The synthetic supF complementation tRNA gene may be isolated from the vector, pVX, which is present in the bacterial strain 39083 available at the American Type Culture Collection. The Sup F sequence as it reads in the polynucleotide vector is antiparallel and comprises:

1 GAATTCTTTC TCAACGTAAC ACTTTACAGC GGCGCGTCAT TTGATATGAT  
GCGCCCCGCT TCCCGATAAG GGACCGAGGCC AGTAAAAGCA TTACCCGTGG  
TGGGGTTCCC GAGCGGCCAA AGGGAGCAGA CTCTAAATCT GCCGTCATCG  
ACTTCGAAGG TTCGAATCCT TCCCCCACCA CCATCACTTT CAAAAGTCCG  
AAAGAATTC (SEQ. ID. NO. 4), and analogs thereof.

Promoters for use in the vector are human promoters or functional portions thereof, promoters derived from or synthesized based on a human promoter, and mammalian homologs of human promoters, or portions thereof. The promoters for use in the polynucleotide vector of the present invention do not encompass viral promoters or viral derived promoters. The human promoters are selected on the basis

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of the tissue and cells to be targeted and should provide optimal expression of the target antigen(s) or antigenic epitopes thereof in the selected mammalian target tissue and mammalian cells. Embodiments of promoters which may be used in constructing the polynucleotide vector of the present invention include but are not limited to the human derived RANTES promoter (Nelson, P.J. et al. J. Immunol. Vol. 151:2601-33, 1993; Ortiz; B.D. et al. Molecular Cell. Biol. pp 202-210, 1996 (Jan); Nelson, P.J. Immunol Vol. 157, No. 3, 1996 (August 1)), truncated RANTES promoters and derivatives thereof. Truncated RANTES promoters include but are not limited to a 249 base pair fragment, a 440 base pair fragment and a 900 base pair fragment. In a preferred embodiment, the truncated RANTES promoter is a base pair fragment of approximately 440 base pairs. A preferred truncated RANTES promoter is a fragment that spans the region approximately between the NCO restriction endonuclease site through the KpnI site of the RANTES promoter as described in GenBank Accession No. S64885. The human derived promoter is selected based on the mammalian target tissue and mammalian target cell in which expression of the target antigen(s) is desired. The target tissue is one containing antigen presenting cells or easily accessible to antigen presenting cells. Such tissue include but is not limited to muscle, lymph nodes, epithelium, subepithelium, skin, and the like. In one embodiment, the promoter is active in muscle or skin. Promoters may express one or more target antigen(s) or antigenic epitope thereof in muscles cells or professional antigen presenting cells. Preferred are promoters which express target antigen(s) in professional antigen presenting cells such as monocytes, macrophages, dendritic cells, Langerhans cells and the like.

The 3' splice sequence and the poly A sequences in the polynucleotide vector are a mammalian sequence, or a synthetic sequence based on a mammalian sequence, preferably a human sequence or mammalian homolog thereof. Possible sources of these elements include but are not limited to bovine growth hormone, human growth hormone gene, and the like. In a preferred embodiment of the polynucleotide vectors the 3' splice (intron) and the poly A sequences are synthetic sequences based on sequences from the human growth hormone gene (DeNoto, F.M. et al. Nuc. Acids. Res. Vol. 9(15):371930, 1981). Oligonucleotides may be synthesized based on the published sequence or modified to condense the third intron and poly A tail sequences

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along with the appropriate splice functions. Poly A-signal sequences which may be used in constructing the polynucleotide vector include but are not limited to:

AATAAA (SEQ. ID. NO. 5)  
ATTAAA (SEQ. ID. NO. 6)  
5 AGTAAA (SEQ. ID. NO. 7)  
AAGAAC (SEQ. ID. NO. 8)  
AATACA (SEQ. ID. NO. 9)

and the like.

In one embodiment, a combined 3' splice sequence and poly A tail sequence for incorporation into the polynucleotide vector construct includes but is not limited to:

10 5' GCCTTAAGGG CCATATGGTG AGTGGATCCC TTGACCCAG GCAGGGATGG  
3' GGAATTCCC GGTATAACAC TCACCTACGG AACTGGGGTC CGCCCTAAC  
15 GGAGACCTG TAGTCAGAGC CCCCGGGCAG CACAGGCCAA TGCCCGTCCT  
CCCTCTGGAC ATCAGTCTCG GGGGCCGTC GTGTCCGGTT ACGGGCAGGA  
20 TCCCCCTGCAG GATGAGTAGT GAGTGCCTCT CCTGGCCCTG GAAGTTGCCA  
AGGGGACGTC CTACTCATCA CTCACGGAGA GGACCGGGAC CTTCAACGGT  
25 CTCCAGTGCC CACCAGCCTT GTCCTAATAA AATTAAGTTG CATCATTTG  
GAGGTACGG GTGGTCGGAA CAGGATTATT TTAATTCAAC GTAGTAAAAC  
TCTGACTAGG TGTCTCTAT AATATTAT 3' (SEQ. ID. NO. 10)  
AGACTGATCC ACAGGAGATA TTATAATA 5' (SEQ. ID. NO. 11)

30 Operably located downstream of the promoter and upstream of the splice and poly A sequences, is the cloning site and stuffer site. The sequence acceptance site is functional in mammalian cells, preferably human cells. The sequence acceptance site is synthetically constructed, however, the sequences are derived from sequences that function in human cells. The sequence acceptance site is designed to directionally accept sequence-specific products from rtPCR-based cloning strategies via unique sequences within the interrupted palindrome recognition sequence for the Bgl I restriction endonuclease, which is incorporated into the PCR primers. The 5' cloning site of the vector was designed in such a way as to provide an integral Kozak consensus sequence (145) and an in-frame initiation codon. Oligonucleotides may be synthesized for the sequence acceptance site so as to provide an initiation codon,

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Kozak consensus sequence and downstream termination codon. Kozak consensus sequence which may be incorporated into the polynucleotide vector includes but is not limited to:

5           GCCCGCC (either A or G) CCAUGG and the like, as are known in the art (Kozak, M. J. Cell Biol. vol. 108, pp 229-241, 1989).

The polynucleotide vector may be constructed to allow for insertion of a internal ribosomal entry site (IRES). Such a site allows for translation of a second open reading frame from a polycistronic mRNA molecule. Such IRES elements are described by Sachs et al., Cell June 13, 1997 Vol. 89: 831-838; Chen, C-Y et al. 10 Science Vol. 268, pp. 415-417, 1995; Tahara, H. et al. J Immunol pp. 6467-6474, 1995; Joshi, C. et al. Nucleic Acids Research Vol. 23, No. 4, pp. 541-549, 1995; Ting, J. et al. DNA Vol. 7, No. 4, pp 275-286, 1988; and Aharon, T. et al. Molecular Cellular Biology, Mar 1993, pp 1971-1980. A polynucleotide vector comprising an IRES allows for translation of more than one target antigen or epitope 15 from the polycistronic mRNA. A polynucleotide vector comprising an IRES allows expression of a target antigen and a cytokine or chemokine from a polycistronic mRNA.

A stuffer site is provided in the vector as a space occupying, non-coding fragment of DNA. A stuffer site may be synthetically synthesized. In one 20 embodiment the stuffer site has the nucleic acid sequence:

CCTCGGTACCTGCCATGGCGCGGATTCTTATCACTGATAAGTTGGTGGACATATTATGTTATCAGTGATAAAAGTGTCAAGCATGACAAAGTTGCAGCCGAATACAGTGATCCGTGCCGCCCTGGACTGTTGAACGAGGTCGGCGTAGACGGTCTGACGACACGCAAACCTGGCGGAACGGTTGGGGTGCAGCAGCCGGCGTTACTGGCACTTCAGGAACAAAGCAGGCGCCTTAAGGGCCATATG 25 CCG (SEQ. ID. NO. 12), and variants thereof.

This stuffer region sequence is based on the stuffer region in vector pCDM8 (In Vitrogen). The sequence of the stuffer is not critical as long as it is one which does not interfere with cloning of the rt-PCR target sequence and does not contain a 30 BglII site. The stuffer site is not present in the final polynucleotide vector vaccine, as it is excised.

In one embodiment of the present invention the polynucleotide vector

comprises pITL as schematically depicted in Figure 1. cDNA encoding one or more target antigen(s) or antigenic epitopes thereof may be ligated into the cloning site or sequence acceptance site depicted in Figure 1. The sequence acceptance site is designed to directionally accept sequence specific products from rtPCR based cloning strategies via unique sites within an interrupted palindrome recognition sequence for a restriction endonuclease which is incorporated into the PCR primer. In one embodiment, the pallidrome recognition sequence is for the Bgl I restriction endonuclease. In a preferred embodiment the sequence acceptance site is as depicted in Figure 2 in which the 5' acceptance site reads on the (+) strand as the sequence 5  
GCCA/CCATGGCc wherein the GCC sequence is recognized by Bgl I and ATG is 10 the start codon. GCc keeps the sequence in-frame and encodes the amino acid serine. 15 The 3' acceptance site reads on the (+) strand as GCCTTAAGGGC.

Any exogenous gene may be inserted into the polynucleotide vector and expressed in a host cell or host tissue. Of interest are exogenous genes which are 15 associated with diseases or pathological conditions in humans. Of particular interest are the antigens or antigenic epitopes thereof which are useful in stimulating an immune response in a mammal, preferably a human for the purpose of prevention or treatment of a disease or pathological condition.

The polynucleotide vaccine vector is useful as a single target antigen vaccine 20 and as a multi-target antigen vaccine. Of interest are target antigens derived from intracellular organisms and the host intracellular compartment. The target antigen includes but is not limited to tumor antigen, bacterial, viral, parasitic antigen and the like. Such antigens include but are not limited to antigens or antigenic epitopes 25 derived from rabies virus, plasmodium, Herpes Virus, HIV 1, HIV 2, influenza virus, HBV, HCV, SIV, Cytomegalovirus, Mycobacterium, Measles virus, papillomavirus, and the like. The polynucleotide vector is particularly well suited for expression of tumor associated genetic derangements, which encompass aberrant transcription 30 regulatory controls on oncogene mutation, a dysregulated developmental or oncofetal gene, a mutated tumor suppressor gene, dysregulated cellular enzymes coding sequences such as metaloproteases or combinations thereof. Tumor antigen or antigenic epitopes thereof which may be expressed by the polynucleotide vector include but are not limited to p53, RB, ras, int-2, Hst, Tre17, BRCA-1, BRCA-2,

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MUC-1, HER-2/neu, PEM/MUC-1, and the like. The polynucleotide vector is also well suited for insertion of one or more target antigens or antigenic epitopes thereof derived for an individual's tumor. The nucleic acid sequences encoding one or more target antigens is ligated into the cloning site of the polynucleotide vector thereby forming the polynucleotide vector vaccine.

The nucleic acid sequence encoding a cytokine and/or chemokine may also be incorporated into the polynucleotide vector vaccine for enhancement of an immune response. Such cytokines include but are not limited to interleukin 2, interleukin 4, interleukin 7, granulocyte-monocyte colony stimulating, factor (GM-CSF), tumor necrosis factor (TNF), interferon, interleukin 12, interleukin 3, interleukin 15, interleukin 8, interleukin 18 and the like. Nucleic acid sequences encoding chemokines which may incorporate into the cloning site of the polynucleotide vector vaccine include those that recruit lymphocytes and antigen presenting cells into a target tissue. These include but are not limited to RANTES, MCP, MIP- $\alpha$ , MIP-1 $\beta$ , defensins and the like.

In one embodiment, the polynucleotide vector vaccine comprises a gene encoding a target antigen or epitope thereof and a gene encoding GM-CSF or functional portion thereof. The polynucleotide vector allows expression of both the target antigen or epitope thereof and GM-CSF in the target cell or target tissue resulting in an enhanced immune response to the target antigen or epitope thereof.

The present invention encompasses methods of making a humanized polynucleotide vector which comprises operably linking an origin of replication with a nucleotide sequence which provides a mechanism for selection, which in turn is operably linked to a human derived promoter or mammalian homolog thereof which is functional in a mammalian target tissue and mammalian target cells. The promoter is operably linked with a cloning site containing a sequence acceptance site which directionally accepts target sequence specific products from rtPCR cloning. The 5' sequence acceptance site comprises an initiation codon and Kozak consensus sequence. The 3' sequence acceptance site comprises termination codons (Figure 2). The method also provides a stuffer region, two separate in1 frame termination codons, a human derived 3' splice (intron) or mammalian homolog thereof and a human derived poly A sequence.

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The present invention also provides a method of preparing a polynucleotide vector vaccine in which one or more nucleic acid sequences encoding one or more target antigens or antigenic epitopes thereof are incorporated. The method of making the substantially humanized polynucleotide vector vaccine provides a product able to express stable mRNA for one or more target sequences.

For vaccination against a tumor in a patient, the construction of the polynucleotide vector vaccine is tailored to the individual's tumor antigen or antigens. Most potential tumor antigens are recognized as peptides derived from intracellular proteins, some of which are dysregulated or mutated oncogene products (74, 8, 9, 30, 53, 71, 132b, 133b, 134b, 135b, 136, 138) as opposed to unique, intact cell surface molecules. Therapies such as monoclonal antibodies or peptide vaccine strategies are generally directed against a single tumor associated antigen and as such are confronted by the fundamental issue of intratumoral antigenic heterogeneity and are limited in their scope of applicability due to both intertumoral antigenic heterogeneity and the nature of most tumor-associated antigens. The multiple and diverse set of molecular events involved in neoplastic transformation, in the evolution to a malignant/metastatic phenotype, and in resistance to therapy provides a foundation for devising novel therapeutic strategies as each molecular event has the potential to generate a tumor specific antigen (139-141). Current molecular techniques and the recently described polynucleotide vaccine strategy (102, 132a, 133a, 134a, 135a, 103) allow a novel antigen-specific, anti-tumor, immunomodulatory therapeutic strategy. Polynucleotide vaccine strategies have been shown in animal models to elicit a broad antigen-specific immune response including humoral, proliferative and cytolytic T cell responses. The present invention addresses the issues of tumor heterogeneity and takes advantage of this phenomena to generate a highly specific yet broad anti-tumor immune response directed against multiple defined tumor-specific antigens.

Using RT-PCR technology, it is possible to isolate target cDNA sequences encoding antigenic polypeptides from individual tumors and to use this nucleic acid template in the polynucleotide vector to induce an immune response to the uniquely mutated or dysregulated intracellular tumor associated proteins in individuals immunized with the vaccine.

mRNA isolated from a patient's tumor is subjected to rtPCR by standard

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techniques. PCR primers are used to amplify, clone into the vector of the present invention and subsequently evaluate, by thermocycle sequencing, the resultant cDNA for mutations. If mutated sequences are identified and present in at least about 20% of clones analyzed, the sequences are used as a component of the polynucleotide vaccine of the present invention. For the oncofetal proteins, such as int-2 and Hst, demonstration of the presence of mRNA is sufficient for constructs with the oncofetal sequences to form part of the polynucleotide vaccine. Tumors which express MUC-1 or HER-2/neu antigens by immunohistochemistry have monomorphic constructs for these two tumor antigens which are ligated into the polynucleotide vector. Thus, each individual patient may receive a unique set of plasmid constructs depending upon the repertoire of mutated oncogenes, mutated tumor suppressor genes, expressed oncofetal genes, and disregulated monomorphic antigens. Examples of such include but are not limited to nucleic acid sequences for c-erb-  $\beta$ -2/HER2/neu, PEM/MUC-1, Int-2, Hst, BRCA-1, BRCA-2, truncated EGFRvIII, MUC-1, CEA, p53, ras, RK, Myc, Myb, OB-1, OB-2, BCR/ABL, GIP, GSP, RET, ROS, FIS, SRC, TRC, WT1, DCC, NF1, FAP, MEN-1, ERB-B1 (*Cell* vol. 64:pp235-326, Jan. 25, 1991) and the like, which are inserted into the polynucleotide vector.

In one embodiment, the polynucleotide vector vaccine of the present invention include but are not limited to: pITL-HER2/neu, pITL-PEM/MUC-1, pITL-Int-2, pITL-Hst, pITL-BRCA-1, pITL-BRCA-2, pITL-p53, pITL-ras, pITL-RB, pITL-TRE17 and the like.

The polynucleotide vector vaccine of the invention may be formulated into a pharmaceutical composition comprising the vector vaccine and a pharmaceutically acceptable carrier such as physiological saline and the like, as known in the formulation art. The pharmaceutical composition may comprise one or more different polynucleotide vectors depending on the needs of the patient to be treated. The pharmaceutical composition may also comprise an agent which enhances the uptake and expression of the polynucleotide vaccine into the target tissue and target cells. These agents include but are not limited to mycotoxic agents. Such mycotoxic agents include but are not limited to dextrose, bupivacaine HCl (1-butyl-N-[2,6-dimethyl-phenyl]-2-piperidine carboxamide) (Sigma, St. Louis, MO), and the like.

The present invention also encompasses methods of stimulating an immune

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response, cellular and humoral, against a target antigen or antigenic epitope with little or no toxicity or autoimmune reactions using the polynucleotide vector vaccine. In the case where the target antigen is a tumor antigen or antigens, the immune response generated by the method should result in generation of cytotoxic T lymphocytes which are capable of inhibiting or killing tumors cells or premalignant cells expressing the tumor antigen(s).

In the method of treatment, at least one polynucleotide vector vaccine comprising a nucleotide sequence of the target antigen or multiple antigens is administered to a patient in a dose of at least about 1  $\mu$ g per component, preferably at doses of about 1  $\mu$ g to about 1 mg per DNA per construct, although higher or lower doses may be used. The dose is administered intramuscularly (IM) or by cutaneous ballistic impregnation in a volume of about 0.1 ml to a volume of about 1 ml. Preferably the volume is about 0.5 ml per injection. In one embodiment, the dose of vaccine is administered at three week intervals for a total of three doses.

In one embodiment of the method of treatment, each intramuscular injection of the polynucleotide vaccine is preceded by IM injection of an expression enhancing agent approximately 24 hours before vaccine administration. The enhancing agent may be bupivacaine-HCl, dextrose, and the like. In the case of dextrose, a concentration of about 10 to about 50% is administered, preferably a concentration of about 50%. Preferably the enhancing agent is bupivacaine-HCl and functional equivalents. Bupivacaine-HCl is administered in a concentration of about 0.25% to about 1.0%, preferably a concentration of about 0.5% in a volume of 0.5 ml.

In another embodiment of the method of treatment, the polynucleotide vector vaccine is administered using gene gun techniques. In this embodiment, the polynucleotide vector vaccine is adsorbed on to particles and administered by bombardment of the particles into the target tissue such as skin. (Yang, N.S., et al. 1990, Proc. Nat'l. Acad. Sci. USA 87: 9568; Williams, R.S. et al. 1991, Proc. Nat'l. Acad. Sci. USA 88:2726; Fynan, E.R.G. et al. Proc. Nat'l Acad. Sci. USA 90:11478; Eisenbraun, M.D. et al. DNA and Cell Bio. 1993 12:791).

Patients may also receive conventional cancer therapy prior to the polynucleotide vaccine therapy. Treatments that induce or result in immunodepression are discontinued a minimum of 4 weeks prior to immunization with the polynucleotide

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vaccine. In the case of viral, bacterial, yeast, parasitic infections, appropriate antiviral, antibiotics, antifungals and the like may be administered prior to, concurrent with, or after administration of the polynucleotide vaccine.

Patients are monitored at periodic intervals to access the efficacy of the treatment. Tumor burden of the individual is monitored. The immune response to each component of each individual's vaccines preparation is evaluated. This is accomplished by evaluating the proliferative and cytolytic response to autologous cells transfected with single individual components (plasmid constructs). Tumor specific CTL<sub>p</sub> frequency is assessed for each patient. Anti-tumor humoral responses are assayed by staining of autologous tumor cells and analysis by fluorescent cell scanning (FACS) or the whole cell ELISA technique. Immune responses induced by polynucleotide vector vaccines containing nucleic acid sequences encoding virus, bacterial, yeast, parasite target antigens may be measured using standard immunoassays as are known in the art.

Polynucleotide vaccine therapy is applicable to any cancer in which a genetic defect is identified. Such cancers include but are not limited to breast cancer, prostate cancer, lung cancer, liver cancer, melanoma, colorectal cancer, pancreatic cancer, thymoma, sarcoma, non-Hodgkins lymphoma, Hodgkins lymphoma, leukemia, ovarian cancer and the like. Of particular interest is breast adenocarcinoma.

The invention further comprises an isolated antibody or antigen binding site thereof elicited by immunization with the polynucleotide vector vaccine. The antibody has specificity for and reacts or binds with the target antigen or antigenic epitope thereof. The antibody may be polyclonal, monoclonal, chimeric antibody or may be single chain antibody produced by genetic engineering. (U.S. Patent No. 4,946,778; Milenic et al Cancer Res. Vol 51:6363-6371, 1991; Shu et al Proc. Nat'l Acad. Sci USA, Vol. 90:7995-7999, 1993; Bird et al Science, Vol. 242:423-426, 1988; Traunecker et al The EMBO J., Vol. 10(12):3655-3659, 1991; Oi et al BioTechniques, Vol. 4, No. 3:214-221, 1986). Monoclonal antibody may be produced by *in vitro* or *in vivo* target antigen stimulation. In one embodiment, target antigen specific B lymphocytes are isolated from a human immunized with a polynucleotide vector vaccine comprising as sequence encoding the target antigen. Human hybridomas are produced by methods known in the art and the monoclonal

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antibody isolated and purified. (Cairns et al J. Clin. Invest., Vol. 74:880-887, 1984; Nakamura et al J. Immunol., Vol. 141, No. 12:4165-4172, 1988).

The antibody and antigen binding site thereof is useful as a therapeutic and also as a diagnostic agent to detect target antigen or antigenic epitope thereof in tissue and biological samples using standard immunoassays. (Yokota, et al Cancer Res., Vol. 52:3402-3408, 1992; Colcher et al Cancer Res., Vol. 48:4597-4603, 1988).

While the invention is described above in relation to certain specific embodiments, it will be understood that many variations are possible, and that alternative materials and reagents can be used without departing from the invention. In some cases such variations and substitutions may require some experimentation, but will only involve routine testing.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying current knowledge, readily modify and/or adapt for various applications such specific embodiments without departing from the generic concept, and therefore such adaptations and modifications are intended to be comprehended within the meaning and range of equivalents of the disclosed embodiments.

All references and patents referred to are incorporated herein by references.

## EXAMPLE 1

### Construction Of The Vector

A plasmid, pITL was designed to contain minimal exogenous DNA and to be maximally "humanized", with elements of the plasmid except the origin of replication and selection element (supF) being derived from human genetic elements. (Figure 1).

Standard molecular techniques were used for the following manipulations. From piVX a 207 base sequence (SupF) is excised using ECO RI and gel purified. This is ligated into the ECO RI site of the cloning vector pBluescript II KS (Stratagene, La Jolla, CA). The minimal colE1 origin of replication (142) is utilized for plasmid replication and growth within permissive strains of *E. coli*. It was excised from pBR327 (ATCC #37516) using Sau 96 and Bfa I enzymes as a double digest and the 453 base pair fragment gel purified. This fragment is 'polished', that is the overhangs from these digests are filled in with DNA polymerase I (Klenow) large

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fragment. This blunt end, 'polished' fragment is ligated into the Sma I site of pBluescript II KS in which the SupF gene has previously been ligated into the ECORI site. The resultant product of these 2 ligation consists of pBluescript KS with the SupF gene and a second ColI origin of replication, both located within the multiple cloning site.

The splice poly A sequence was synthesized and inserted into the vector pGEM 7f+ between the Hind II and Xho I sites.

The unique directional cloning site or sequence acceptance site is upstream of two separate in frame termination codons, a 3-prime splice (intron), and poly-A sequences (the latter two synthetic sequences based on the human growth hormone gene) (144). These synthetic sequences were directionally inserted into pGEM-7F at the Xho and HindIII sites (Genosys, The Woodlands, TX). This vector is digested with Bam HI and HIND III. Likewise, the product from the minimal colE1, SupF, pBluescriptII KS is digested with these same enzymes. These two resultant purified, double digests are ligated together. The product of this ligation contains pGEM7F+ with the synthetic splice poly A sequence, minimal col E1 origin of replication and SupF gene all with the multiple cloning site.

The stuffer region is isolated from pCDM8 by using the following PCR primers to amplify an approximately 200 base pair portion of the stuffer (the exact PCR product will be 253 bases but, incorporates sequence modifications as detailed below). The upper primer (positive strand or 5') is

CCTCGGTACCTGCCACCATGGCGCGGATTCTTTAT (SEQ. ID. NO. 13) and spans the 2217 to 2252 bases of pCDM8 but, diverges at 2235 to accommodate linking restriction sites in this case the 5' Bgl I cloning site (Figure 2) and a Kpn I site at the upstream, 5' end. This is important later for promoter ligation. The lower primer (negative strand or 3') is CGGCATATGGCCTTAAGGCGCCGCTTGTTCCTGAAGT (SEQ. ID. NO. 14) and spans the 2394 to 2433 bases of pCDM8 but, diverges at 2454 to accommodate linking restriction sites in this case the 3' Bgl I cloning site (see Figure 2) and a Nde I site at the upstream, 5' end. This allows isolation of a fragment without a Bgl I site or any other conflicting sites. This fragment is digested with Nde I and gel purified. The pGEM product from above is digested with Nde I (site engineered into synthetic

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sequence) and Stu I which results in a blunt end on the 5' end. The fragment isolated above is ligated into this digested construct, i.e., Nde I end to Nde I end and blunt to blunt.

The following is the splice poly A signal sequence cloned into the Xho and Hind III sites of pGEM-7Zf reading from Xho site to Hind III site. Bold is intron 3, underline is poly A signal sequence, double underline is cloning site and potential site for internal ribosomal entry sites (IRES), with the remainder from the fifth exon but, following the stop codon:

**gCCTTAAGggCCATATggTgAgTggATgCCTTgACCCCCAggCggggA**  
10           TgggggAgACCTgTAgTCAGAgCCCCCgggCAgCACAggCCAATg  
          CCCGTCCTTCCCCTgCAggATgAgTAgTgAgTgCCTCTCCTggCCC  
          TggAAgTTgCCACTCCAgTgCCCACCAgCCTTgTCCTAATAAAA  
          TTAAgTTgCATCATTgTCTgACTAggTgTCCTCTATAATATTA  
          T (SEQ. ID. NO. 15)

15 All triplets in italics are stop codons. After processing of punitive mRNA, excision of intron 3, the sequence will contain duplicate stop codons in two reading frames. The 3rd reading frame can contain a stop codon by mutagenizing the double underlined sequence resulting in deletion of last "g" of the double underlined sequence.

20 The RANTES promoter construct has a Kpn I site at its 3' end and depending upon the size of the truncation, various restriction sites at the 5' end. From pGL-RANTES the 249 base fragment is excised using Kpn I and Sac I enzymes. The product of the above paragraph is likewise digested with Kpn I and Sac I. The two digests, 249 bases and 1176 bases respectively, are gel purified and ligated together resulting in the intact vector pITL-1.

## EXAMPLE 2

### Construction Of Minimal Promoter Elements For Expression In Skeletal Muscle, Monocytes And Dendritic Cells

30 The choice of promoter sequence included in the vectors is critical as the promoter must be functional in the mammalian target tissues preferably human tissues and drive the expression of the mRNA at an appropriate level. The human RANTES

promoter is one promoter useful in the present invention because it is smaller than the CMV or b-actin promoters, is functionally between the two in terms of activity, and is functional in the desired tissues (146). This promoter is highly conserved between human, rat and mouse (146, 147). This promoter has been well characterized and contains myoD like elements functional in skeletal muscle cells along with elements known to be active in cells of the reticuloendothelial system. The minimal promoter elements necessary for expression in lymphocytes has been determined (146, 147). Constructs with a series of 5' truncations of the RANTES promoter upstream of the luciferase reporter are constructed in order to evaluate the minimal promoter elements required for transcriptional activity in cells of interest. Such truncated RANTES promoters include a 249,440 and 990 base pair fragments.

Primary cultures of skeletal muscle cells were purchased (Clonetics, San Diego, CA) and cultured according to manufacturers directions. These cells can be transiently transfected with the control plasmid pGreen Lantern (Gibco, BRL, Gaithersburg, MD) using the cationic lipid mixtures Lipofectin and Lipofectamine (Gibco, BRL, Gaithersburg, MD) with only slight modifications from the manufacturer's suggested protocol. These modifications include trypsinizing cells and replanting 20 minutes before transfection and exposing the cells to the lower concentrations of cationic lipids. The previously described pGL-RANTES promoter constructs, pGL-basic and pGL-control vectors are transfected similarly and luciferase activity is assayed after 72 hours using the Luciferase Assay System (Promega, Madison, WI). To control for transfection efficiency pCMV- $\beta$ Gal (Clontech, San Francisco, CA) is cotransfected with the above plasmids and assayed using Galacto-light Plus (Tropix Inc., Bedford, MA). Luminescence is determined for both assays on an existing LKB scintillation counter.

Monocytes are isolated by elutriation from normal donor leukapheresis packs. The elutriation protocols provides an 90-95% pure monocyte population as evidenced by FACS analysis for CD1-a, CD3, CD14, CD16, CD19, CD45RO, CD56, CD80, HLA-DR and HLA-DQ. The remaining 5% of cells are granulocytes/basophils. FACS analysis of MHC class II and CD25 expression reveals that these cells are not activated after the isolation procedure. Routinely  $5 \times 10^8$  monocytes are isolated from a given leukapheresis pack. The same series of constructs and assay conditions are

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evaluated using these cellular preparation. The transfection procedure are modified for suspended cells, but utilize cationic lipid mixtures are above.

The recently reported ability to isolate dendritic cells (DCs), defined by phenotype and functional assays, from peripheral blood mononuclear cells (148) makes it possible to assay for promoter activity in this cellular population. Following elutriation, monocytes are placed into culture with IL-4 and GM-CSF as reported by Sallusto and Lanzavecchia (148). After 48 hours a representative sample is evaluated by FACS for DC phenotype, i.e., CD3-, CD19-, CD14-, CD1a-c+, CD80+, HLA-DR+, and HLA-DQ+. Upon demonstration of DC phenotype, cationic lipid transient transfections are carried out as above with identical assay procedures followed.

Upon conclusion of these experiments the appropriate promoter fragment is determined based on the activities in the above three cellular preparations. Preferred, is a promoter which drives optimal expression in muscle cells, or antigen presenting cells of the target antigen(s). The promoter fragment is ligated into the pITL vector. This constitutes the base vector. The vector is transfected using the standard heat shock method into frozen competent DH10  $\beta$ /p3 *E. coli* bacteria (Gibco BRL, Gaithersburg, MD), grown under ampicillin and tetracycline selection as previously described. The cloning site of the base vector is the location of all subsequent cloning of reporter gene sequences or target antigen sequences.

The computer generated nucleic acid sequence which approximates the sequence of one base vector, pITL, is as follows:

TGCCATGGCG	CGGATTCTTT	ATCACTGATA	AGTTGGTGGA	CATATTATGT
TTATCAGTGA	TAAAGTGTCA	AGCATGACAA	AGTTGCAGCC	GAATACAGTG
25 ATCCGTGCCG	GCCCTGGACT	GTTGAACGAG	GTCGGCGTAG	ACGGTCTGAC
GACACGAAA	CTGGCGGAAC	GGTTGGGGGT	GCAGCAGCCG	GCGCTTTACT
GGCACTTCAG	GAACAAGCGG	GCGCCTTAAG	GGCCATATGG	TGAGTGGATG
CCTTGACCCC	AGGCGGGGAT	GGGGGAGACC	TGTAGTCAGA	GCCCCCGGGC
AGCACAGGCC	AATGCCCGTC	CTTCCCCCTGC	AGTGAGTAGT	GACTGCCCGG
30 GTGGGATCCC	TGTGACCCCT	CCCCAGTGCC	TCTCCTGGCC	CTGGAAGTTG
CCACTCCAGT	GCCCACCAGC	CTTGTCTCAA	TAAAATTAAG	TTGCATCATT
TTGTCTGACT	AGGTGTCCCTC	TATAATATTA	Taagcttgat	atcgAATTCT
TTCTAACGT	AACACTTTAC	AGCGGCGCGT	CATTTGATAT	GATGCGCCCC

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	GCTTCCCGAT	AAGGGAGCAG	GCCAGTAAAA	GCATTACCG	TGGTGGGTT
	CCCGAGCGGC	CAAAGGGAGC	AGACTCTAAA	TCTGCCGTCA	TCGACTTCGA
	AGGTTCGAAT	CCTTCCCCCA	CCACCATCAC	TTTCAAAAGT	CCGAAAGAAT
	Tcctgcagcc	CGTAGGCCG	TAGTTAGGCC	ACCACTTCAA	GAACTCTGTA
5	GCACCGCCTA	CATACTCGC	TCTGCTAATC	CTGTTACCAG	TGGCTGCTGC
	CAGTGGCGAT	AAGTCGTGTC	TTACCGGGTT	GGACTCAAGA	CGATAGTTAC
	CGGATAAGGC	GCAGCGGTG	GGCTGAACGG	GGGGTTCGTG	CACACAGCCC
	AGCTTGGAGC	GAACGACCTA	CACCGAACTG	AGATAACCTAC	AGCGTGAGCA
	TTGAGAAAGC	GCCACGCTTC	CCGAAGGGAG	AAAGGGGAC	AGGTATCCGG
10	TAAGCGGCAG	GGTCGGAACA	GGAGAGCGCA	CGAGGGAGCT	TCCAGGGGGA
	AACGCCTGGT	ATCTTTATAG	TCCTGTCGGG	TTTCGCCACC	TCTGACTTGA
	GCGTCGATT	TTGTGATGCT	CGTCAGGGGG	CGGGAGCCTA	TGGAAAAACG
	CCAGCAACGC	GGCCggggga	tccggaGAGC	TCACTCTAGA	TGAGAGAGCA
	GTGAGGGAGA	GACAGAGACT	CGAATTTCG	GAGCTATTTC	AGTTTTCTTT
15	TCCGTTTGT	GCAATTTCAC	TTATGATACC	GGCCAATGCT	TGGTTGCTAT
	TTTGGAAACT	CCCCTTAGGG	GATGCCCTC	AACTGGCCCT	ATAAAGGGCC
	AGCCTGAGCT	GCAGAGGATT	CCTGCAGAGG	ATCAAGACAG	CACGTGGACC
	TCGCACAGCC	TCTCCCACAG	GTACC	1425 base pairs (SEQ. ID. NO. 16),	
					and variants thereof.

20                  The minimal ColE1 origin of replication comprises base pairs 712 through 1164 of pITL, the SupF sequence comprises base pairs 495 through 701, the minimal RANTES promoter comprises base pairs 1177 through 1425, the stuffer sequence comprises base pairs 1 through 221 and the combined splice and poly A sequences comprises base pairs 222 through 481. Base pairs 482 through 494, 602 through 611, and 1165 through 1176 are extraneous, noncoding sequences derived from plasmids from which the component sequences were excised from (sequences in lower case in pITL sequence).

25

An enzyme restriction map of pITL is provided in Figure 5.

30

### EXAMPLE 3

#### Purification Of Vector

The vector, pITL, requires the presence of the p3 helper plasmid for appropriate selection and subsequent isolation. However, the p3 helper plasmid is not part of the therapeutic product. Standard plasmid DNA isolation techniques cannot

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selectively isolate pITL (1.5 - 2.0 kb) from the much larger p3 plasmid (60kb). To avoid administering a contaminating plasmid as part of the polynucleotide vaccine preparation, a separate isolation procedure is established based on anion exchange chromatography. Anion exchange resins have a higher affinity for large DNA molecules than small molecules at any given NaCl concentration. The 30 fold larger p3 plasmid will be retained at a NaCl concentration in the elution gradient which releases the pITL. After standard alkaline/SDS lysis of large volume culture, digestion with RNase A at 100ug/ml, and isopropanol precipitation the resultant pellet is resuspended in TE and applied to the anion exchange column. Small disposable Nucleobond AX columns (Nest Group Inc., Southborough, MA) or MonoQ column (Pharmacia Biotech, Piscataway, NJ) are run on an FPLC system (Pharmacia Biotech, Piscataway, NJ) with a NaCl gradient from 0.01M to 3M. The covalently closed circular (CCC) form of the pITL construct is eluted at less than 1M NaCl while the p3 plasmid is retained. The open circular (OC) form of the pITL plasmid is eluted at a lower NaCl concentration in the gradient and is discarded. Columns are used only once to avoid cross contamination. The resultant retained (CCC) fractions are precipitated with ethanol at -20°C. The pellet is resuspended with sterile PBS and stored aseptically at-20°C until use. Purity of the preparation is assessed by agarose electrophoresis and ethidium bromide staining of undigested and HindIII digested samples. Hind III digests linearize both pITL and the p3 helper plasmid. An ultraviolet spectra across the 240nm to 320 nm range is also obtained on an existing DU 65 UV/Vis spectrophotometer (Beckman, Fullerton, CA). Sample aliquots are retained for microbiological evaluation as needed in the animal experiments. The polynucleotide vector of the present invention is greater than 95% pure, preferably greater than 99% pure, more preferably greater than 99.9% for use in humans.

#### EXAMPLE 4

##### Evaluation of the kinetics of expression of a reporter sequence from the plasmid pITL in an Animal Model

A rat model using Fisher 344 rats is used to evaluate the polynucleotide vaccine vector. The rat model was chosen over mouse to avoid the complication of

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the mouse mammary tumor virus which is present in most mouse mammary tumor lines and which can function as a superantigen causing significant alterations in the immune system. Cohorts of 45 rats, vaccinated with a given dose of DNA are used, for evaluation of toxicity and target sequence expression over a period of 60 days.

5 Three individual members of each cohort are sacrificed and submitted for necropsy on days; 0, 1, 2, 3, 5, 7, 14, 21, 28, 35, 42, 49, and 56 in order to evaluate sequence expression and toxicity. Techniques of polynucleotide vaccination have been described previously (102, 132a, 133a, 134a, 107, 119). All animals receive intramuscular injections using standard sterile technique and 27 gauge needles however, in an effort  
10 to more accurately mimic the clinical situation the muscle is not surgically exposed. The lateral limb of individual animals is clipped, as needed, and cleansed with 70 % ethanol prior to injections. On day # -1 the left and right gastrocnemius muscle is injected with 200 microlitres of 0.5% bupivacaine-HCL and 0.1% methylparaben in isotonic NaCl ( 0.5% Marcaine, Sanofi Winthrop Pharmaceuticals or 0.5 %  
15 Sensorcaine, Astra USA, Inc.). The injection site is identified by a cutaneous tattoo, < 1 mm diameter, with sterile Indian Ink (autoclaved 20 min.) placed at the time of bupivacaine injection. On Day 0 the closed circular DNA preparation in a volume of 200 microlitres of injection grade normal saline is injected into one limb only at the same site as the bupivacaine injection as noted by the tattoo. The opposite limb will  
20 be used as a control with an equal volume of sterile injection grade normal saline, without any DNA, administered in the same fashion as above.

25 The "humanized" nucleic acid sequence encoding a green fluorescent protein sequence portion or variant thereof present in the Green Lantern vector (Gibco BRL, Gaithersburg, MD) is utilized. This vector results in production of a green fluorescent protein in human skeletal muscle cells in vitro and in vivo. This reporter protein has the advantage of not requiring any treatment of tissue to visualize expression and it is stable in both frozen section and formalin fixed paraffin embedded tissues. Using fluorescent microscopy trace expression of this protein is accurately identified using conditions used for evaluation of FITC labeled antibodies. Therefore, this reporter also provides the opportunity to evaluate reticuloendothelial cells by FACS to evaluate  
30 possible uptake and expression of the reporter vector. PCR primers to the green lantern protein are used with the appropriate Bgl I 5' extensions to amplify and clone

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the cDNA encoding green fluorescent protein into pITL, in a manner identical to that used to insert tumor target antigen sequences.

The construct containing the green fluorescent protein, pITL-GFP, is sequenced by standard dideoxy sequencing techniques to confirm the fidelity of the cloning process. The nucleic acid sequence for GFP target sequence contained in pITL is as follows:

ATGAGCAAG	GGCGAGGAAC	TGTTCACTGG	CGTGGTCCA	ATTCTCGTGG
AACTGGATGG	CGATGTGAAT	GGGCACAAAT	TTTCTGTCAG	CGGAGAGGGT
GAAGGTGATG	CCACATACGG	AAAGCTCACC	CTGAAATTCA	TCTGCACCAC
10 TGGAAAGCTC	CCTGTGCCAT	GGCCAACACT	GGTCACTACC	TTCACCTATG
GCGTGCAGTG	CTTTTCCAGA	TACCCAGACC	ATATGAACGA	GCATGACTTT
TTCAAGAGCG	CCATGCCCGA	GGGCTATGTG	CAGGAGAGAA	CCATCTTTT
CAAAGATGAC	GGGAACCTACA	AGACCCGCGC	TGAAGTCAAG	TTCGAAGGTG
15 ACACCCCTGGT	GAATAGAACATC	GAGTTGAAGG	GCATTGACTT	TAAGGAAGAT
GGAAACATTC	TCGGCCACAA	GCTGGAATAC	AACTATAACT	CCCACAATGT
GTACATCATG	GCCGACAAGC	AAAAGAATGG	CATCAAGGTC	AACTTCAAGA
TCAGACACAA	CATTGAGGAT	GGATCCGTGC	AGCTGGCCGA	CCATTATCAA
CAGAACACTC	CAATCGGCGA	CCGCCCTGTG	CTCCTCCCAG	ACAACAATTA
20 CCTGTCCACC	CAGTCTGCC	TGTCTAAAGA	TCCCAACGAA	AAGAGAGACC
ACATGGTCCT	GCTGGAGTTT	GTGACCGCTG	CTGGGATCAC	ACATGGCATG
GACGAGCTGT	ACAAGTGAGC	(SEQ. ID. NO. 17), and analogs thereof.		

The pITL-GFP DNA is prepared as described above, for the vaccination procedure. In one embodiment, the computer generated sequence which approximates the full pITL-GFP sequence comprises:

Tatgagcaaggcgaggaactgttcaactggcgtggtcccaattctcgtaactggat  
ggcgatgtgaatggcacaaattttctgtcagcggagagggtgaagggtatgccacat  
acggaaagctcaccctgaaattcatctgcaccactggaaagctccctgtgccatggccaa  
30 cactggtcactacccatggcgtgcagtgtttccagataccagaccatat  
gaagcagcatgactttcaagagcgcattgcggagggctatgtgcaggagagaacc  
atcttttcaaagatgacggaaactacaagaccccgctgaagtcaagttcgaaagggt  
acaccctggtaatagaatcgagttgaagggattgactttaaggaagatggaaacat  
35 tctcgccacaagctggaaataactataactcccacaatgtgtacatcatggccgac  
aagcaaaaagaatggcatcaaggtaactcaagatcagacacaacattgaggatggat

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ccgtgcagctggccgaccattatcaacagaacactccaatcgcgacggccctgtct  
cctcccagacaaccattacctgtccaccaggctctgcccgtctaaagatccaaacgaaa  
agagagaccacatggtctgtggagtttgaccgctgtggatcacacatggcat  
ggacgagctgtacaagttagc**CATATGGTGAGTGGATGCCTGACCCCAGGCAGGGAT**  
5 GGGGGAGACCTGTAGTCAGAGCCCCCGGGCAGCACAGGCCAATGCCGTCTTCCCT  
GCAGTGAGTAGTGACTGCCGGGTGGATCCCTGTGACCCCTCCCCAGTGCCTCTCCT  
GCCCTGGAAGTTGCCACTCCAGTGCCACCAGCCTGTCTTAATAAAATTAAAGTTGC  
ATCATTTGCTGACTAGGTGTCTCTATAATATTaagcttgcatacgAATTCTTT  
10 CTCAACGTAACACTTACAGCGCGGTCTTGTATGATGCGCCCCGCTTCCCGAT  
AAGGGAGCAGGCCAGTAAAGCATTACCGTGGTGGGTTCCCGAGCGGCCAAAGGGA  
GCAGACTCTAAATCTGCCGTATCGACTTCGAAGGTTGAATCCTTCCCCACCACCA  
TCACCTTCAAAAGTCCGAAAGAATTcctgcagcccgtagccgtAGTTAGGCCACCA  
CTTCAAGAACTCTGTAGCACCCTACATACCTCGCTCTGCTAATCCTGTTACCAAGTG  
15 GCTGCTGCCAGTGGCGATAAGTCGTCTTACGGGTTGGACTCAAGACGATAGTTAC  
CGGATAAGGCGCAGCGGTGGCTGAACGGGGGTTCGTGCACACAGCCCAGCTTGG  
GCGAACGACCTACACCGAACTGAGATACTACAGCGTGAGCATTGAGAAAGCGCCACG  
CTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGTAAGCGCAGGGTCGGAACAGGAG  
AGCGCACGAGGGAGCTCCAGGGGAAACCGCTGGTATCTTATAGTCCTGTCGGGTT  
20 TCGCCACCTCTGACTTGAGCGTCGATTTGTGATGCTCGTCAGGGGGCGGAGCCTA  
TGGAAAAACGCCAGCAACGCGGCCgggggatccggaGAGCTCACTCTAGATGAGAGAG  
CAGTGAGGGAGAGACAGAGACTCGAATTCCGAGCTATTCAAGTTCTTCCGTT  
TTGTGCAATTCACTTATGATACCGCCAATGCTGGTTGCTATTGGAAACTCCCC  
25 TTAGGGGATGCCCTCAACTGGCCCTATAAAGGGCCAGCCTGAGCTGCAGAGGATTCC  
TGCAGAGGATCAAGACAGCACGTGGACCTCGCACAGCCTCTCCCACAGGTACC (SEQ  
ID NO. 18) and analogs thereof. The nucleic acid sequence encoding the green  
fluorescent protein sequence are in lower case and bolded.

Toxicity from polynucleotide vaccination, as reported in the literature (102, 132a, 133a, 134a, 135a, 103, 104-131), has been limited to minimal local inflammatory responses at the site of injection. Expression of the target protein, GFP, is expected through 60 days. The literature supports extended expression, upwards of 18 months, with other conventional vectors.

Cohort #1 consists of 45 rats each 200 microlitres of normal saline without DNA. Cohort #2 consists of 45 rats each receiving 1 microgram of pITL-GFP closed circular plasmid DNA. Cohort # 3 consists of 45 rats each receiving 10 micrograms of pITL-GFP closed circular plasmid DNA. Cohort # 4 consists of 45 rats each

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receiving 100 micrograms of pITL-GFP closed circular plasmid DNA. Cohort # 5 consists of 45 rats each receiving 1 milligram of pITL-GFP closed circular plasmid DNA.

Three animals from each of the cohorts # 1 through 5 are euthanized on day # 0, 1, 2, 3, 5, 7, 14, 21, 28, 35, 42, 49, and 56, either by inhaled carbon dioxide or cervical dislocation. Animals are examined at necropsy immediately following euthanasia. The following tissue specimens are submitted for histologic evaluation by H & E stained sections: skeletal muscle and skin from both injection sites, normal skin and skeletal muscle, inguinal lymph node(s), thymus, bone marrow (femur), spleen, gastrointestinal tract with Peyer's patches, heart, lung, liver, kidney, brain/spinal cord, and any other tissues which appear grossly abnormal at the time of necropsy. Tissues from these same organs are prepared and frozen in OCM media for frozen section evaluation of GFP expression by fluorescent microscopy and stored at -70°C for future studies. Blood specimens not to exceed 2 ml are collected by retro-orbital plexus or tail vein phlebotomy no more than weekly for evaluation of hematologic parameters.

If distant or significant local toxicity is detected at any of the DNA dose levels using the pITL-GFP, additional cohorts of 45 animals is treated identically but, administered pITL (without the reporter sequence) at identical dose levels to pITL-GFP and at all subsequent increased dose levels. If no toxicity is seen at any level a single cohort, #5a, 45 additional rats will receive the highest DNA dose level of pITL to confirm the absence of vector derived toxicity.

#### EXAMPLE 5

##### Evaluation of the impact of tumor presence on the kinetics of polynucleotide vaccine expression, pITL

Previous work by Ochoa and colleagues has suggested that in tumor bearing mice there is a profound immune deficit characterized by T cell receptor and signal transduction defects (149). In light of these observations, the presence of tumor might alter the stability and expression by the polynucleotide vaccine. It is possible that these defects will be manifest in this model and may result in alterations of the

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immune response to the reporter protein. This maybe manifest by increased and persistent expression or a skewing of the immune response with a predominance of antibodies and subsequent ADCC or immune complex deposition. Therefore, these parameters are evaluated in tumor bearing animals. The syngeneic rat mammary tumor line 13762 (ATCC #1666-CRL) which is known to express rat HER2/neu is utilized.

Cohorts # 6 through 10, 30 rats per cohort, involve animals with established 13762 mammary tumors. Rats are injected with  $5 \times 10^5$  cells into the subcutaneous space 14 days prior to receiving polynucleotide vaccination. All rats receive an identical vaccine sequence as that described above. Cohort #6 receive 200 microlitres of normal saline without DNA. Cohort #7 receive 1 microgram of pITL-GFP closed circular plasmid DNA. Cohort #8 receive 10 micrograms of pITL-GFP closed circular plasmid DNA. Cohort #9 receive 100 micrograms of pITL-GFP closed circular plasmid DNA. Cohort #10 receive 1 milligram of pITL-GFP closed circular plasmid DNA.

Three animals from each of the cohorts #6 through 10 are euthanized on Day # 3, 7, 14, 21, 28, 35, 42, 49, and 56 or earlier if animals become moribund due to tumor growth. Animals are necropsied immediately after euthanasia. Tissue specimens are identical to cohorts #1 through 4 with the exception that sections of the tumor nodule is submitted for H&E stained histochemistry and a specimen is frozen as above. Blood specimens not to exceed 2 ml is collected by retro-orbital plexus or tail vein phlebotomy no more than weekly for evaluation of hematologic parameters. Clearly, if tumor growth is either accelerated or sustained this will cause morbidity to the animals.

#### EXAMPLE 6

##### Evaluation of immune response and toxicities to anti-tumor vaccination, using constructs with partial sequences of rat HER2/neu, in normal and tumor bearing rats

The C-erbB-2 or HER-2/neu gene product, originally isolated from neuroblastoma tumor lines, is homologous to the epidermal growth factor receptor (150-154) and expressed in up to 40% of breast carcinomas (26). Since the original

characterization of this sequence the amplification and overexpression of the protein has been variably associated with prognosis in both ovarian and breast carcinomas (155-165). In murine cell lines, activation of HER-2/neu has been associated with mutations in the transmembrane domain. However, to date most tumor specimens demonstrate gene amplification rather than point mutations (155, 157, 158, 166).  
5 HER2/neu amplification occurs in up to one third of breast carcinomas. Monoclonal antibodies recognizing the c-erbB-2 or HER2/neu protein have been shown to be cytostatic in vitro for cell lines overexpressing the c-erbB-2 or HER2/neu protein (167-170). Additionally, CTL lines isolated from patients with ovarian carcinoma  
10 have been shown to recognize synthetic peptides corresponding to two regions on the HER2/neu protein (74) despite the fact that c-erbB-2 has been reported to be expressed in the aerodigestive and urologic tract (171). These findings suggest that HER2/neu can be a source of tumor antigens for an anti-tumor immune response.

The choice of HER2/neu as the initial target, tumor-associated, antigen is predicated upon considerations of the animal model and the human clinical trial. A concern of polynucleotide vaccination is the theoretical induction of an autoimmune phenomena to related normal proteins. HER2/neu shares the high degree of homology with other normal cellular proteins, i.e. with other members of the EGFR family (150, 154) as shown in Figure 3. Therefore, a phase 1 clinical trial with this target antigen is most likely to expose this potential toxicity. The human Her2/neu target antigen corresponds with amino acid residues in Figure 3. Additionally, it has been demonstrated that the rat mammary tumor 13762 expresses rat neu. To minimize the potential for autoimmune phenomena a limited partial sequence, including the transmembrane domain (the site of the activating mutation in rat neu), and a segment  
20 of the cytoplasmic domain with limited homology to EGFRs is constructed using rtPCR techniques from rat 13762 and the human breast cancer cell line SK-BR-3 (ATCC # HTB-30). These sequences share very limited homology with other EGFR members. Identical sections of the rat and human sequence are utilized. The target  
25 amino acid sequence for both human Her2/neu and rat Her2/neu is depicted in Figure  
30 4.

The nucleic acid sequence encoding the partial human Her 2/neu target sequence is ligated at the initiation site of the polynucleotide vector. In one

embodiment, the nucleic acid sequences encode at least one or a combination of the following amino acid sequences or portion thereof of Her 2/neu:

PDLSYMPIWKF	PDEEGACQPC	PINCTHSCVD	LDDKGCPAEQ
RASPLTSIIS	AVVGILLVVV	LGVVFGIL (SEQ. ID NO. 19), or portion	
or mammalian homolog thereof and,			
PAPGAGGMVH	HRHRSSSTRS	GGGDLTLGLE	PSEEEAPRSP
LAPSEGAGSD	VFDGDLGMGA	AKGLOSLPTH	DPSPLQRYSSE
DPTVPLPSET	DGYVAPLTCS	PQPEYVNQPD	VRPOPPSPRE
GPLPAARPAG	ATLERPKTLS	PGKNGVVKDVF	FAFGGAVENP
EYLTPQGTCS	PQPEYVNQPD	VRPQPPSPRE	GPLPAARPAG
ATLERPKLSP	GKNGVVKDVF	AFGGAVENPE	YLTPQGGAAP
QPHPPPAFSP	AFDNLYYWDO	DPPERGAPPS	TFKGTPTAEN
PEYLGLDVPV (SEQ. ID NO. 20), or portion or mammalian homolog thereof.			

In another embodiment, the nucleic acid sequences encode at least one region corresponding to the transmembrane domain of Her 2/neu comprising the amino acid sequence:

IISAVVGILLVVVLGVVFGILI (SEQ. ID NO. 21), or portion or mammalian homolog thereof.

The respective nucleic acid sequences encoding the target antigen are ligated into pITL. Subsequent clones are sequenced by standard dideoxy sequencing techniques (Sequenase, USB/Amersham, Arlington Heights, IL) to assure fidelity of the insert. Appropriate clones of the vector are transformed into DH10 b/p3, amplified, purified, and characterized as described above. These preparations are used in the following animal experiments.

Cohorts #11 through 15, 20 rats per cohort, receive three repeated injections in the same limb, every three weeks, administered exactly as described above with the exception that the tattoo is placed only once. The polynucleotide vaccine preparation consist of pITL-rHER2/neu, i.e., pITL with a partial rat HER2/neu cDNA sequence. Two weeks after the final injection the rats are challenged with  $5 \times 10^6$  13762 tumor

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cells in no more than 0.5 ml sterile normal saline administered into the subcutaneous space. Cohort #11 receives only sterile saline and no closed circular plasmid DNA per injection. Cohort #12 receives 1 microgram of pITL-rHER2/neu closed circular plasmid DNA per injection. Cohort #13 receives 10 micrograms of pITL-rHER2/neu closed circular plasmid DNA per injection. Cohort #14 receives 100 micrograms of pITL-rHER2/neu closed circular plasmid DNA per injection. Cohort #15 receives 1 milligram of pITL-rHER2/neu closed circular plasmid DNA per injection. Cohorts #16 through #20, 20 rats per cohort, have  $5 \times 10^5$  cells in no more than 0.5 ml sterile normal saline administered into the subcutaneous space 10 to 14 days prior to initiating a polynucleotide vaccine sequence of three injections administered every three weeks so as to have an established tumor not to exceed 1.0 cm in diameter. Cohort #16 receives only sterile saline and no closed circular plasmid DNA per injection. Cohort #17 receives 1 microgram of pITL-rHER2/neu closed circular plasmid DNA per injection. Cohort #18 receives 10 micrograms of pITL-rHER2/neu closed circular plasmid DNA per injection. Cohort #19 receives 100 micrograms of pITL-rHER2/neu closed circular plasmid DNA per injection. Cohort #20 receives 1 milligram of pITL-rHER2/neu closed circular plasmid DNA per injection.

Animals from cohorts #11 through 15 have venous blood samples obtained pre-vaccine initiation, at the time of each vaccination, at the time of tumor challenge, and every three weeks thereafter. These samples are used to assess humoral immune responses by FACS analysis on both 13762 tumor cells and RAT2 cells transiently transfected with the vaccine construct, pITL-rHER2/neu, using cationic lipid techniques as described above for promoter evaluation. A 'sandwich' ELISA is also used to evaluate the development of anti-HER2/neu antibodies, i.e. using test serum as the capture antibody, application of either SKBR3 (HER2/neu + human breast cancer cell line) or 13762 cellular lysates, detection with anti-rat or anti-human HER2/neu antibody (Ab-4 and Ab-2 or Ab-5 respectively, Oncogene Sciences) and followed by an appropriate anti-isotope enzyme labeled detection antibody and calorimetric determination.

Three individual animals from each cohort are euthanized at the following time points: at the time of tumor challenge, two weeks after tumor challenge, four weeks after tumor challenge, six weeks after tumor challenge, and the remaining animals

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when moribund or no later than one year after tumor challenge. Euthanized animals have spleens harvested for isolation of T lymphocytes, tumor nodule sampling for histochemical examination (both fixed and frozen section), and necropsied with gross abnormalities directing further tissue section submission. Splenic T lymphocytes are isolated used to assay tumor specific CTL precursor frequency using limiting dilution assays (89) and signal transduction defects as previously described for mouse model systems (149, 172).

Animals from cohorts #16 through #20 have venous blood samples obtained pre-tumor inoculation, pre-vaccine initiation, at the time of each vaccination, and every three weeks thereafter. These samples are used to assess humoral immune responses as described above. Three individual animals from each cohort are euthanized at the following time points: at the time of first vaccination (pre treatment), at the time of second vaccination, at the time of the third vaccination, 3 weeks after completion of vaccination, 6 weeks after completion of the vaccination sequence, 12 weeks after completion of the vaccination sequence, and the remaining animals when moribund or no later than one year after completion of the vaccination sequence. Euthanized animals have spleens harvested for isolation of T lymphocytes, tumor nodule sampling for histochemical examination (both fixed and frozen section), and necropsied with gross abnormalities directing further tissue section submission. Splenic T lymphocytes are isolated used to assay tumor specific CTL precursor frequency and signal transduction defects.

#### EXAMPLE 7

Evaluation of toxicity of anti-tumor  
vaccination with constructs containing  
partial sequences of human HER2/neu in rats

Cohorts #21 through 25, 45 rats per cohort, receive three repeated injections in the same limb, every three weeks, administered exactly as described above with the exception that the tattoo is placed only once. The construct used is the base vector with the human partial HER2/neu sequence construct, pITL-hHER2/neu. The nucleic acid sequence of one embodiment of a pITL-hHer2/neu polynucleotide vector vaccine

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comprises:

GCCACCATGGCCcctgaccctccatgcacatgtggaaatggccatgtggatgaggagggcgcatgccagcc  
ttggcccatcaactgcacccactctgtgtggaccctggatgacaaggcgccccgcccgcagcagagagccagcc  
ctgacgtccatcatctgcggtggtggcattctgtgtggatgtggatggatggatccatcaagcg  
acggcagcagaagatcacatgtccagacccgtccccggcgctggggcatggtccaccacaggcaccgcagc  
atctaccaggagtggcggtgggacactgacactagggctggagccctgtgaagaggaggccccaggctccactg  
gcacccicgaaggggctggctccgatgtttgtatggatgggacactggaaatggggcagccaaggggctgcaaagcc  
tccccacacatgaccccageccctacagcggtaacgtgaggacccacagtacccctgcctcagactgtatggc  
tagtgtccccctgacctgcagccccagccctgaataatgtgaaccagccagatgttcggccccagcccc  
tcgccccgagaggccctgcctgtgcggcaccgtgggtccactgtggaaaggccaaagactctcccccagg  
gaagaatgggttgtcaaagacgttttgccttgggggtccgtggagaaccccgagacttgcacacccaggag  
gagctgcccccteagccccacccctctgcctcagcccttcgacaaccttattactggaccaggaccac  
cagagcggggggctccacccagcaccctcaaagggacacctaaggcagagaacccagagttggatggac  
gtgcagtgtgaaGCCTTAAGGGCCAATGGTGAGTGGATGCCTGACCCCAGG  
CGGGGATGGGGAGACCTGTAGTCAGAGCCCCGGCAGCACAGGCCAA  
TGCCCGTCTTCCCTGCAGTGAGTAGTGAATGCCGGGTGGATCCCTG  
TGACCCCTCCCCAGTGCCTCTCCTGGCCCTGGAAGTTGCCACTCCAGTGC  
CCACCAGCCTGTCTAATAAAATTAAAGTTGCATCATTTGTCTGACTAGG  
TGTCTCTATAATATTATaatcgAATTCTTCTCAACGTAACACTTTA  
CAGCGGCGCGTCATTGATATGATGCGCCCCGCTTCCGATAAGGGAGCA  
GGCCAGTAAAGCATTACCCGTGGTGGGGTCCCGAGCGGCCAAAGGGA  
GCAGACTCTAAATCTGCCGTCATCGACTTCGAAGGTTCGAATCCTCCCC  
CACCACCATCACTTCAAAGAAACTCTGTAGCACCGCCTACATACCTCGCTC  
GTTAGGCCACCACTCAAGAAACTCTGTAGCACCGCCTACATACCTCGCTC  
TGCTAATCCTGTTACCAGTGGCTGCCAGTGGCGATAAGTCGTGTCTT  
ACCGGGTTGGACTCAAGACGATAGTTACCGATAAGGCGCAGCGGTGG  
GCTGAACGGGGGGTTCGTGCACACAGCCCAGCTGGAGCGAACGACCTAC  
ACCGAACTGAGATAACCTACAGCGTGAGCATTGAGAAAAGCGCCACGCTTCC  
CGAAGGGAGAAAGGCGGACAGGTATCCGTAAGCGGCAGGGTCGGAACA  
GGAGAGCGCACGAGGGAGCTCCAGGGGAAACGCCCTGGTATCTTATAG  
TCCTGTCGGGTTCGCCACCTCTGACTTGAGCGTCGATTTGTATGCTC  
GTCAGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCgggggatcc

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5 ggaGAGCTCACTCTAGATGAGAGAGCAGTGAGGGAGAGACAGAGACTCGA  
ATTTCGGAGCTATTCAGTTTCTTCCGTTGTGCAATTCACTTATG  
ATACCGGCCAATGCTGGTTGCTATTTGGAAACTCCCCTAGGGATGC  
CCCTCAACTGGCCCTATAAAGGGCCAGCCTGAGCTGCAGAGGATTCTGC  
AGAGGATCAAGACACGTGGACCTCGCACAGCCTCTCCCACAGGTACCT  
(SEQ ID NO. 22) and analogs thereof. The nucleic acid sequence encoding the  
human Her 2/neu target sequence are in lower case and bolded.

10 Three animals from each of the cohorts # 21 through 25 are euthanized on Day  
# 0, 1, 2, 3, 5, 7, 14, 21, 28, 35, 42, 49, and 56, either by inhaled carbon dioxide  
or cervical dislocation. Animals are examined at necropsy immediately following  
euthanasia. The following tissue specimens are submitted for histologic evaluation by  
H & E stained sections: skeletal muscle and skin from both injection sites, normal skin  
and skeletal muscle, inguinal lymph node(s), thymus, bone marrow (femur), spleen,  
15 gastrointestinal tract with Peyer's patches, heart, lung, liver, kidney, brain/spinal  
cord, and any other tissues which appear grossly abnormal at the time of necropsy.  
Tissues from these same organs are prepared and frozen in OCM media for frozen  
section evaluation if indicated and stored at -70°C for future studies. Blood specimens  
not to exceed 2 ml are collected by retro-orbital plexus or tail vein phlebotomy no  
more than weekly for evaluation of hematologic parameters.

20

#### EXAMPLE 8

##### Phase I Trial

25 The Phase I study is designed to determine the maximal tolerated dose of a  
polynucleotide vaccine, evaluate potential toxicities, and immunological effect of the  
polynucleotide vector vaccine.

#### VACCINE PREPARATION

30 pITL plasmid vector and constructs containing the various target sequences are  
detailed herein. Individual plasmid constructs have been sequenced to confirm target  
sequence fidelity. These vectors are transfected using the standard heat shock method  
into frozen competent DH10  $\beta$ /p3 E. Coli. bacteria (Gibco BRL, Gaithersburg, MD),

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grown under ampicillin and tetracycline selection as previously described. The vector pITL requires the presence of the p3 helper plasmid for appropriate selection and subsequent isolation. However, all current plasmid DNA isolation techniques cannot selectively isolate pITL (1.5 - 2.0 kb) from the much larger p3 plasmid (60kb). To avoid administering a contaminating plasmid as part of the polynucleotide vaccine preparation a separate isolation procedure is disclosed herein based on anion exchange chromatography. Anion exchange resins have a higher affinity for large DNA molecules than small molecules at any given NaCl concentration. The 30 fold larger p3 plasmid is retained at a NaCl concentration in the elution gradient which releases the pITL.

After standard alkaline/SDS lysis of large volume culture, digestion with RNase A at 100ug/ml, and isopropanol precipitation the resultant pellet is resuspended in TE and applied to the anion exchange column. Small disposable Nucleobond AX columns (Nest Group Inc., Southborough, MA) or MonoQ column (Pharmacia Biotech, Piscataway, NJ) run on an FPLC system (Pharmacia Biotech, Piscataway, NJ) are used with a NaCl gradient form 0.01M to 3M. The covalently closed circular (CCC) form of the pITL construct is eluted at less than 1M NaCl while the p3 plasmid is retained and the open circular (OC) form of the pITL plasmid is eluted at a lower NaCl concentration in the gradient and is discarded. Columns are used only once to avoid cross contamination. The resultant retained fractions are precipitated with ethanol at -20°C. The pellet is resuspended with sterile PBS and stored aseptically at -20°C until use. Purity of the preparation is assessed by agarose electrophoresis and ethidium bromide staining of undigested and Hind III digested samples. Hind III digests linearizes both pITL and the 3 helper plasmid. An ultraviolet spectra across the 240nm to 320nm range is also used. Sample aliquots are retained for microbiological evaluation as needed.

#### STUDY DESIGN

The patients have histopathological confirmation of the diagnosis of adenocarcinoma of the breast. The specimen submitted to pathology is evaluated for surface expression of HER-2/neu immunohistochemistry. If tumor is available for

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biopsy the immunotherapy laboratory receives 2 cm<sup>3</sup> (minimum of 1 cm<sup>3</sup>) of fresh tumor transported on ice. The sample is used to generate tumor cell and autologous normal breast epithelium or normal fibroblast cell cultures and for mRNA isolation for rtPCR analysis.

5 All patients receive intramuscular (IM) injections (vaccine or control). The vaccine consists of the Her 2/neu polynucleotide vaccine construct as detailed herein at the appropriate dose level per cohort, resuspended in 0.5 ml injection grade, sterile, normal saline and administered in the lateral quadriceps with the patient in the supine position. Cohort #1 receives three initial IM injections of normal saline control (no  
10 DNA) prior to receiving three polynucleotide vaccinations.

All intramuscular injections are preceded by IM injection of 0.5 ml. 0.5 % Bupivacaine 24 hours before vaccine administration.

The injection site is marked with a sterile indian ink pinpoint tatoo at the time of the Bupivacaine administration.

15 Cohort #1  
Patients receive IM bupivacaine followed by IM injection of sterile saline x 3 at three week intervals. Subsequently, patients receive a dose of 100 microgram of basal plasmid DNA, pITL without the Her 2/neu target sequence insert, per vaccination administered in the contralateral lower extremity at three week intervals x 3.  
20

The first three patients have a muscle biopsy of the sterile saline injection site at week 12. The last three patients have a muscle biopsy of the basal plasmid injection site three weeks after the last vaccination, i.e. week 21.

25 In this cohort alone, if no toxicity is identified, the patients are vaccinated with the pITL-Her 2/neu construct, in the lower extremity originally injected with bupivacaine and saline. The dose of the pITL-Her 2/neu construct is the highest dose at which two patients have been vaccinated at least twice without observed toxicity greater than grade 2.

30

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Cohort #2

The initial vaccination consists of 1 microgram of the reporter plasmid, pITL with the PCR amplified GFP sequence. Patients receive a dose of 1 micrograms of pITL-Her 2/neu DNA per vaccination administered in the contralateral lower extremity at three week intervals x 3. Groups of 2 patients have a muscle biopsy of the reporter plasmid vaccination site at weeks 3, 6 and 9 respectively.

5-

Cohort #3

The initial vaccination consists of 10 micrograms of the reporter plasmid, pITL with the PCR amplified GFP sequence. A dose of 25 micrograms pITL-Her 2/neu DNA per vaccination is administered in the contralateral lower extremity at three week intervals x 3. Groups of 2 patients have a muscle biopsy of the reporter plasmid vaccination site at weeks 3, 6, and 9 respectively.

10

15

Cohort #4

The initial vaccination consists of 25 micrograms of the reporter plasmid, pITL with the PCR amplified GFP sequence. A dose of 100 micrograms pITL-Her 2/neu DNA per vaccination is administered in the contralateral lower extremity at three week intervals x 3. Groups of 2 patients have a muscle biopsy of the reporter plasmid vaccination site at weeks 3, 6, and 9 respectively.

20

Cohort #5

The initial vaccination consists of 100 micrograms of the reporter plasmid, pITL with the PCR amplified GFP sequence. A dose of 1000 micrograms pITL-Her 2/neu DNA per vaccination is administered in the contralateral lower extremity at three week intervals x 3. Groups of 2 patients have a muscle biopsy of the reporter plasmid vaccination site at weeks 3, 6, and 9 respectively.

25

30

All patients are evaluated for anti-tumor responses at the conclusion of the vaccination sequence, every two months x 3, then every three months. Patients free from progression at 6 months are eligible for repeated vaccination sequences identical to that initially administered minus the initial vaccination with the reporter plasmid.

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## EVALUATION

Medical history, T lymphocyte function, fluorescent cell sorting, antigen specific responses are performed as detailed in Example 9 below.

5

## DOSE ADJUSTMENT AND MODIFICATIONS

The maximally tolerated dose (MTD) of total DNA per vaccination is defined as that dose at which 0 or 1/6 patients experience polynucleotide vaccine related dose limiting toxicity (DLT) with the next higher dose level provoking DLT in any 2 patients from that cohort.

10

If dose limiting toxicity is observed in the first two patients of any cohort, the dose is reduced by 50% and the equivalent of a full cohort enrolled at this reduced dose unless dose limiting toxicity again encountered at this new dose.

15

## **EXAMPLE 9**

### **Phase I/II Evaluation Of Polynucleotide Vaccination In Advanced Breast Cancer**

20

#### **Polynucleotide Vaccine Preparation**

25

The patients undergo an excisional biopsy with histopathological confirmation of the diagnosis of adenocarcinoma of the breast. The specimen submitted to pathology is also used for evaluation of surface expression of HER-2/neu and MUC-1 by immunohistochemistry. The immunotherapy laboratory receives 2 cm<sup>3</sup> (minimum of 1 cm<sup>3</sup>) of fresh tumor transported on ice. This specimen is divided with 50% of the sample dispersed with collagenase and the resultant cellular preparation cryopreserved for use as described below. 10-15% of the sample is used to generate tumor cell and autologous normal breast epithelium or normal fibroblast cell cultures. The remainder is used for mRNA isolation and vaccine preparation with an immediately adjacent slice/section held for histologic confirmation and correlation. The sample is homogenized in Trizol solution (Gibco BRL) for isolation of total RNA. Messenger RNA is isolated with oligo dT agarose (Pharmacia) and subjected

30

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to rtPCR with first strand reverse transcription initiated from oligo d(T)12N using StrataScript Rnase H- Reverse Transcriptase (Stratagene) and the high fidelity pfu (Stratagene) thermostable polymerase for the PCR reaction. (Chapter 15 in: Current Protocols in Molecular Biology, Vol. 2; Ausubel, F.M. et al (eds) John Wiley & Sons, Inc. 1995).

5.

Specific PCR primers for the individual transcripts of interest are used in individual PCR reactions using an aliquot of the cDNA from the reverse transcription of the mRNA. An embodiment of rtPCR primers for the target sequence p53 is as follows:

10

p 53 rtPCR primer 1: g tctgccacca tggcctactc ccc~~t~~gc (SEQ. ID. NO. 23) and variants thereof;

p 53 rtPCR primer 2: ttc ttgggtgacc tacctcttcg gaattgcgcga gtc (SEQ. ID. NO. 24) and variants thereof.

15

The location of the primer extension on the P53 cDNA using primer 1 and primer 2 may be depicted as follows:

20

aaaacclaccaggcagctacggttccgcgtggcgttgtcatttggcacagccaa

## glossary

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gtctgtgacttgcacglactccctgcccctaacaagaatgttttgccaaactggccaaagacc  
tgcccttgtcagctgtgggttgttccacacccccggccggcaccccgctccgcgcctatg  
gccatctacaaggcagtcacagcacatgacggagggttgtgaggcgctgccccccaccatgag  
cgctgtcagatagcgatggctggcccttcagcgcttataccgagtggaaggaaat  
ttgcgttgtggatatttgtatgacagaaacacttttcgacatagtgtgggttgtgcctat  
gagccggcttgggttgtgcactgttaccaccaatccactacaactacatgtgtaaacagt

30

tcctgcattggcgccalgaaccggaggcccaacctcaccatcatcacactggaagaccc  
agtggtaatctactgggacggaaacagctttaggtgcgttttgtgcctgtccctgggaga  
gaccggcgacagaggaaagagaatctccgcaagaaggggagccctaccacgagctgcc  
ccagggagcactaagcgagcacgtccccacaacaccagctccctccccagccaaag  
aagaaaccactggatggagaataatccaccccttcagatccgtggcgtaggccttcgatgt

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~~-ttcttggtgacc taccttcgt~~ gaatigccga gtc

(primer 2)

ttccgagaggc tgaatgaggc ctggaaactc aaggaa~~gccc~~ aggccggaa ggagccagg

gggagcaggg ctcactccag ccacctgaag tccaaaagg gtcagtcac ctccgcct

aaaaaactca tggtaagac agaaggcct gactcagac (SEQ. ID. NO. 25)

Primers from cDNA of ras, RB1, BRCA-1, and TRE17 which incorporate the underlined mutated sequences as shown above for the p53 primers allow the target sequences to be ligated into the polynucleotide vector.

The amplified fragments is digested with the restriction endonuclease Bgl I and  
10 ligated, using T4 DNA ligase, into a similarly digested pITL vector which has been dephosphorylated with calf alkaline phosphatase. Ligation products is electroporated into DH10/p3 bacteria and selected on LB ampicillin and tetracycline plates. Ten to twenty individual colonies from each ligation/transfection are randomly selected and subjected to PCR-based sequencing. The nucleic acid sequences from clones positive  
15 for mutations are used in preparing a polynucleotide vector vaccine. In one embodiment, a mutation from germline is considered a true mutation and selected for use if present in 20% or greater of the colonies evaluated. If two or more mutations meet the above criteria all clones considered valid is used in the polynucleotide vaccine preparation. Individual clones are isolated from the strain DH10/p3 of *E. coli*  
20 using standard molecular biological techniques.

The plasmid of interest is separated from the p3 helper plasmid, required for selection, via ion exchange chromatography using the DEAE 4000-7 resin of the Nest Group Inc. An aliquot of each component of the vaccine preparation is analyzed by agarose gel electrophoresis and ethidium bromide visualization to assure purity of  
25 DNA. An aliquot of these large scale preparations is evaluated for nucleotide sequence to confirm the inserted target sequence. Individual aliquots of plasmid preparations is stored at -20°C as an ethanol precipitate (under 70% ethanol in water). Prior to use, these precipitates are resuspended at a concentration of 1 milligram per milliliter (mg/ml) in sterile, injection grade, normal saline. Appropriate volumes are  
30 diluted as required to match the individual and total dose parameters of each cohort.

All patients receive intramuscular (IM) injections (vaccine or control). The vaccine consist of individual polynucleotide vaccine construct mixtures at the

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appropriate dose level per cohort, resuspended in 0.5 ml injection grade, sterile, normal saline and administered in the lateral quadriceps with the patient in the supine position. Cohort #1 receives three initial IM injections of normal saline control (no DNA) prior to receiving three polynucleotide vaccinations.

5. All intramuscular injections are preceded by IM injection of 0.5 ml. 0.5 % Bupivacaine 24 hours before vaccine administration. The injection site are marked with a sterile indian ink pinpoint tatoo at the time of the bupivacaine HCl administration.

10 Cohort #1

Patients receive IM bupivacaine HCl followed by IM injection of sterile saline x 3 at three week intervals.

- 15 Subsequently, patients receive full compliment polynucleotide vaccination, a total dose of 1 microgram of DNA per construct per vaccination with a total dose of DNA not to exceed 5 micrograms per injection, is administered in the lower extremity at three week intervals x 3.

Cohort #2

- 20 The initial vaccination consists of 10 micrograms of the control plasmid, pITL without any PCR amplified sequences.

Full compliment polynucleotide vaccination, a total dose of 10 micrograms of DNA per construct per vaccination with a total dose of DNA not to exceed 50 micrograms per injection, is administered in the contralateral lower extremity at three week intervals x 3.

- 25 The first three patients have a muscle biopsy of the control plasmid injection site at week three. The second three patients have a muscle biopsy of the control plasmid injection site at week 6. The final four patients have a muscle biopsy of the control plasmid injection site at week 9.

30 Cohort #3

The initial vaccination consists of 10 micrograms of the reporter plasmid, pITL with the PCR amplified GFP sequence.

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Full compliment polynucleotide vaccination, a total dose of 25 micrograms of DNA per construct per vaccination with a total dose of DNA not to exceed 125 micrograms per injection, will be administered in the contralateral lower extremity at three week intervals x 3.

5        The first three patients have a muscle biopsy of the control plasmid injection site at week three. The second three patients have a muscle biopsy of the control plasmid injection site at week 6. The final four patients have a muscle biopsy of the control plasmid injection site at week 9.

10      Cohort #4

Full compliment polynucleotide vaccination, at a total dose of 100 micrograms of DNA per construct per vaccination with the total dose of DNA per injection not to exceed 500 micrograms, is administered in one lower extremity at three week intervals x 4.

15      All patients are evaluated for anti-tumor responses at the conclusion of the vaccination sequence, every two months x 3, then every three months. Patients free from progression at 6 months are eligible for repeated vaccination sequences identical to that initially administered.

20      Freshly excised tumor is used not only to generate individual patient polynucleotide vaccine preparation but, also for the establishment of tumor cell lines using previously described techniques (173-175) and cryopreserved to evaluate tumor specific CTLp. Normal skin fibroblasts or normal autologous breast epithelium derived from the excisional biopsy is used in transfection studies for analysis of immune responses to individual vaccine components. In the absence of established cultures of autologous fibroblasts or breast epithelium, autologous PBMC is used in the same fashion although, transfection procedures require non-specific activation and will be adjusted accordingly.

25      Patients of cohort #1 have leukapheresis performed pre-treatment, after the first three injections (just prior to first polynucleotide vaccination), at the first post treatment follow-up visit, and at the 6 month time point. Patients of cohorts #2, #3 and #4 have leukapheresis performed pre-treatment, at the first post treatment follow-up visit, and at the 6 month time point. Manual leukapheresis of not less than

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100 cc is performed for the purpose of evaluation of tumor-specific CTLp and peripheral blood mononuclear cell phenotype at one year after initiating treatment in all surviving patients.

5        T Lymphocyte Function

The parameters monitored are as follows:

1.      z chain, lck, and fyn proteins by western blot,
2.      nuclear binding factors for  $\gamma$ IFN and NFkB probe sequences,
3.      Lymphokine production (IL-2, IL-4, IL-6, IL-10, and  $\gamma$ IFN), and
4.      Tumor specific CTL precursors (evaluated by limiting dilution assay)

10

Fluorescent Activated Cell Sorting (FACS)

15        The phenotype of peripheral blood mononuclear cells is evaluated by flow cytometry from pretreatment, pre-third vaccination, first post-vaccination follow-up visit, and 6 month samples. The cell markers to be determined are; CD3, CD4, CD8, CD16, CD25, CD56, TCR  $\alpha/\beta$ , TCR  $\gamma/\delta$ , CD45 RO, CD45 RA, CD20.

20        Antigen Specific Responses

25        Non-malignant autologous cell cultures, either fibroblast or breast epithelium depending upon materials obtained from the excisional biopsy, is transiently transfected with a mixture of pITL-GFP and an individual pITL-target sequence construct. Either lipofectin or other established transfection procedures (176) are used. A control transfection of pITL-GFP is utilized for background, non-target sequence, immune responses while the mixture assures that transfection efficiency can be controlled for.

30        Standard proliferation assays using  $^3$ H-thymidine incorporation (177) are performed using PBMC from individual time points. PBMC is placed into culture with irradiated (10-20cGy) transfected cells as stimulators and after 48,  $^3$ H-thymidine is added for a 16 hour pulse. Cells is harvested and counted in the standard fashion.

35        The frequency of CTL precursors (CTLp) for any individual component is expected to be on the order of 1/10-6 (178) and therefore to assess changes in the CTL compartment isolated T cells from PBMC preparations are non-specifically

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stimulated with OKT3 and provide IL-2 for one week. At the conclusion of this period the bulk culture are divided and assayed for cytotoxicity on the individually transfected autologous cells noted above along with K562 as a control for NK activity.

5 Tumor specific CTL precursors are analyzed using irradiated (10-20cGy) autologous tumor cells as stimulators in limiting dilution cultures of PBMC from individual time points, using a previously established technique (178). Standard cytotoxicity assays again using autologous tumor cells and K562 as target cells, are scored and tumor specific CTLp frequency calculated.

10 Muscle biopsies is performed as out patient minor surgery procedures. The tissue samples is multiply divided cross sectionally and separated in an alternating fashion into two groups; the first for standard formalin fixation with H&E histologic examination along with fluorescent microscopy on unstained sections for detection of GFP, the second for frozen section examination and use for immunohistochemical staining. Standard post biopsy care with adequate analgesia and wound care is  
15 administered.

#### CRITERIA FOR RESPONSE

##### Complete Response

20 Disappearance of all clinical and laboratory signs and symptoms of active disease for a minimum period of four weeks. Persistently abnormal bone scans, if stable for a period of not less than 8 weeks is allowed.

##### Partial Response

25 A minimum of 50% reduction in the sum of the bidimensional product of measurable lesions persisting for a minimum period of four weeks. Although every lesion need not demonstrate a full 50% reduction in bidimensional product, no lesion may grow nor can there be new lesions demonstrated in this category of response.

##### Stable Disease

30 No change or decrease in the sum of the bidimensional product less than 50% persisting for a minimum period of four weeks. No lesion may grow and no new lesions can appear in this category of response.

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**Progressive Disease**

25% increase in the sum of the bidimensional product or the appearance of new lesions.

**5 TOXICITY**

Toxicities is graded according to the NCI Common Toxicity Criteria.

**DOSE ADJUSTMENT AND MODIFICATIONS**

The maximally tolerated dose (MTD) of total DNA per vaccination is defined as that dose at which 0 or 1/6 patients experience polynucleotide vaccine related dose limiting toxicity (DLT) with the next higher dose level provoking DLT in any 2 patients from that cohort.

If dose limiting toxicity is observed in the first two patients of any cohort, the dose is reduced by 50% and the equivalent of a full cohort is enrolled at this reduced dose unless dose limiting toxicity is again encountered at this new dose.

If an individual component at a given dose is suspected to be responsible for undue toxicity due to its association with toxicities in separate patients, it is decreased to the previously tolerated dose or dropped from the vaccine preparation if no tolerated dose is previously documented.

If the patient has sufficient potential tumor antigenic templates identified so as to exceed the total dose limitations, if all constructs were to be used, the constructs included in the vaccine preparation is prioritized as follows: 1.) constructs expressing mutated sequences isolated from the patient's tumor, 2.) sequences for Int-2 and Hst and 3.) muc-1 and c-erbB-2/HER2/neu.

25

**REMOVAL OF PATIENTS FROM PROTOCOL**

A. All three of the following, which preclude polynucleotide vaccine production:

1.) No detectable genetic deviation from germline in p53, H or K ras, RB, BRCA-1, TRE2/TRE17,

30 2.) Absence of overexpression of c-erbB-2/HER 2/neu

3.) No expression of MUC-1

B. Progressive disease

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- C. Unacceptable toxicity  
D. Intercurrent Illness which prevents further administration of the treatment  
E. Decision of the patient to withdraw from the study  
F. General or specific changes in the patient's condition which render the patient unacceptable for further treatment in the judgement of the investigator.

5

#### EXAMPLE 10

##### Construction of pITL-1

10

In this embodiment, the polynucleotide vector comprises a 440 base pair region from the RANTES promoter in combination with a 635 base pair region of colE1 origin of replication. The polynucleotide vector was constructed as follows:

15

(A) Splice & poly A sequence was synthesized and inserted into the vector pGEM between the Hind III and Xho I site forming pGEM ELN.

(B) The stuffer region was isolated from pCDM8 by PCR amplification of an approximately 200 base pair portion and blunt end ligated into Xho I site of pGEM ELN forming pGEM ELN/Stuffer.

(C) A 440 base pair region of the RANTES promoter was excised from pGL RANTES using Xho I and Nco I. This fragment is klenow filled (polished) and blunt end ligated into pGEM ELN at the Hind III and Nsi site forming pGEM ELN/RANTES.

20

(D) The 440 base pair region of the RANTES promoter was next excised from pGEM ELN/RANTES using Nsi and Hind III and Inserted into pGEM ELN/Stuffer at its analogous site forming pGEM/ELN/Stuffer/RANTES.

25

(E) A 635 base pair region of the colE1 origin of replication was excised from pBR327 using Bst YI and Ava I. This fragment was polished and blunt end ligated into pBluescript/Sup F forming pBluescript/Sup F/colE1.

30

(F) Sup F was excised from  $\pi$ VX using Eco RI and ligated into the Eco RI site of pBluescript forming pBluescript/Sup F.

(G) The Sup F and colE1 region was removed from pBluescript/ Sup F/colE1 using Hind III and Bam HI and inserted into pGEM/ ELN/Stuffer/RANTES at its analogous site forming pGEM/pITL-1.

(H) pITL-1 is separated from pGEM by excising with Kpn I. pITL-1 is then religated.

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The resulting vector is designated herein as pITL-1. The pITL-1 vector contains the fragment of the RANTES promoter that corresponds with the region in genomic RANTES promoter from about NCO site through the KpnI site. The plasmid polynucleotide vector in *Escherichia Coli* strain DH 10 $\beta$ /P3 was deposited under terms of the Budapest treaty with the American Type Culture Collection, 12301 Parklawn Drive, Rockville MD 20852 USA on April 9, 1997 under ATCC Designation 98400.

5 The computer generated sequence which approximates the sequence of pITL-1 comprises the sequence:

10 GgtacctgccaccaTGGCGCGGATCTTTATCACTGATAAGTTGGTGGACATATT  
ATGTTTATCAGTGATAAAAGTGTCAAGCATGACAAAGTTGCAGCCGAATAC  
AGTGATCCGTGCCGGCCCTGGACTGTTGAACGAGGTCGGCGTAGACGGTC  
TGACGACACGCAAACCTGGCGAACGGTTGGGGTGCAGCAGCCGGCGCT  
TTACTGGCACTTCAGGAACAAGCAGGGCGCctaaggccaaTATGGTGAGTGGAT  
GCCTTGACCCCAGGGGGGGATGGGGGAGACCTGTAGTCAGAGCCCCCGG  
15 GCAGCACAGGCCAATGCCCGTCCTTCCCGTGCAGGATgAGTgagtgcctcct  
gGCCGtGGAagttgccactccagtgCCcaccagecctgtccataaaaaattaaaggatgtcatcatttgtctgacttaggtgt  
cctctataatattataagcttgatatacgAAATTCTTTCGGACTTTGAAAGTGTAGGGTGG  
GGGAAGGATTCGAACCTTCGAAGTCGATGACGGCAGATTAGAGTCTGCT  
20 CCCTTGGCCGCTCGGAACCCCCACCACGGTAATGCTTTACTGGCCTG  
CTCCCTTATCGGGAAAGCGGGGCGCATCATATCAAATGACGCGCCGCTGTA  
AAGTGTACGTTGAGAAAGAATTCCtgcagccccGCCGCGTTGGCGTTTTC  
CATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCA  
25 GAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTCCCCCTG  
GAAGCTCCCTCGTGCCTCTCCTGTTCCGACCCCTGCCGCTTACGGATAC  
CTGTCCGCTTTCTCCCTCGGAAGCGTGGCGTTCTCAATGCTCACGC  
TGTAGGTATCTCAGTTGGTGTAGGTCGTTGCTCCAAGCTGGGCTGTGTG  
CACGAACCCCCCGTTGACGCCGACCGCTGCCCTTATCCGTAACTATCG  
30 TCTTGAGTCCAACCGTAAGACACGACTATGCCACTGGCAGCAGCCA  
CTGGTAACAGGATTAGCAGAGCGAGGTATGTTAGGCGGTGCTACAGAGTTC  
TTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTGGTAT  
CTGCGCTGCTGAAGCCAGTTACCTTCGGAAAAAAGAGTTGGTAGCTCTT  
GATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTGTGCAAG  
35 CAGCAGATTACGCGCAGAAAAAAAGGATCTggggatccggagagctccaaacgcgttgg  
atgcattggatggggaaaggaggtaagatcgttaatgaataagcaggaaacttgaagactcagtgactcagtgatataaaa  
gactcagtgacttcgtatccctgtccactgcctgttgtcccaagaaagcggctccgtctctgaggaggaccctt  
ccctggaaaggtaaaactaaggatgtcagcagagaaaattttccacattggcgttggcaaaagaggaaactgtGAGC  
TCACTCTAGATGAGAGAGCAGTGAGGGAGAGACAGAGACTCGAATTCC  
40 GGAGCTATTTCAGTTCTTCCGTTGTGCAATTCACTATGATACC  
GGCCAATGCTTGGTGTATTTGGAAACTCCCCTAGGGGATGCCCTC  
AACTGGCCCTATAAAGGGCCAGCCTGAGCTGAGAGGATTCCCTGCAGAGG  
ATCAAGACAGCACGTGGACCTCGCACAGCCTCTCCAC (SEQ ID NO. 28)

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It can be appreciated by those in the art that the actual nucleic acid sequence of the ATCC deposited plasmid may vary slightly from the computer generated sequence due to slight variations in the ligation sites of the various components that comprise the vector. Such variations will not affect the functioning of the vector.

5 The actual sequence of the deposited vector including the ligation sites may be performed using routine sequencing methods as are known in the art.

A smaller polynucleotide vector was constructed, differing from pITL-1 by about 200 base pairs, was constructed as described above with the exception that a base pair region of approximately 220 base pairs of the RANTES promoter was used.

10 The resulting vector is referred to herein as pITL-A. This smaller vector in *E. coli* strain DH-10 $\beta$ /P3 was deposited under terms of the Budapest treaty with the ATCC on April 9, 1997 under ATCC designation 98401. The computer generated sequence which approximates the sequence of pITL-A comprises the sequence:

15 GgtacctgccaccaTGGCGCGGATTCTTATCACTGATAAGTTGGTGGACATATT  
ATGTTTATCAGTGATAAAAGTGTCAAGCATGACAAAGTTGCAGCCGAATAC  
AGTGATCCGTGCCGCCCTGGACTGTTGAACGAGGTCGGCGTAGACGGTC  
TGACGACACGCAAACCTGGCGAACGGTTGGGGGTGCAGCAGCCGGCGCT  
TTACTGGCACTTCAGGAACAAGCGGGCGCcitaagggccaTATGGTGAGTGGAT  
GCCTTGACCCCCAGGCCGGGATGGGGGAGACCTGTAGTCAGAGCCCCCGG  
GCAGCACAGGCCAATGCCGTCTTCCCCTGCAGGATgAGTTagtgagtgccttcct  
20 gGCCtGGAagttggccactccagtgCCcaccagccctgtcttaataaaatataagttgcattttgtctgacttaggtgt  
cctctataatattataagttgatatcGAATTCTTCGGACTTTGAAAGTGTGGTGG  
GGGAAGGATTCGAACCTTCGAAGTCGATGACGGCAGATTAGAGTCTGCT  
25 CCCTTGGCCGCTCGGGAACCCCACCACGGTAATGCTTTACTGGCCTG  
CTCCCTTATCGGGAAAGCGGGCGCATCATATCAAATGACGCCGCTGTA  
AAGTGTACGTTGAGAAAGAATTCCtgcagcccGCCGCGTTGCTGGCGTTTC  
CATAGGCTCCGCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCA  
30 GAGGTGGCGAAACCCGACAGGACTATAAAGATAACCAGGCCTTCCCCCTG  
GAAGCTCCCTCGTGCCTCTCCTGTTCCGACCCCTGCCGCTTACCGGATAC  
CTGTCGCCCTTCTCCCTCGGAAGCGTGGCGTTCTCAATGCTCACGC  
TGTAGGTATCTCAGTTGGTAGGTCGTCCTCCAAGCTGGCTGTGTG  
CACGAACCCCCCGTTAGCCGACCCGCTGCCCTTACCGGTAATATCG  
35 TCTTGAGTCCAACCCGTAAGACACGACTTATGCCACTGGCAGCAGCCA  
CTGGTAACAGGATTAGCAGAGCGAGGTATGTTAGGCGGTGCTACAGAGTTC  
TTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTGGTAT  
CTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTT  
GATCCGGCAAACAAACCAACCGCTGGTAGCGGTGGTTTTTGTGCAAG  
40 CAGCAGATTACGCCAGAAAAAGGATCTggggatccggagagtcACTCTAGA  
TGAGAGAGCAGTGAGGGAGAGACAGAGACTCGAATTCCGGAGCTATTTC  
AGTTTCTTCCGTTGTGCA ATTCACTTATGATACCGCCAATGCTT

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GGTTGCTATTGGAAACTCCCTTAGGGATGCCCTCAACTGGCCCTAT  
AAAGGGCCAGCCTGAGCTGCAGAGGATTCCCTGCAGAGGATCAAGACAGC  
ACGTGGACCTCGCACAGCCTCTCCCACA  
(SEQ ID NO. 27)

5

### EXAMPLE 11

#### pITL-1-GFP

The target reporter gene encoding green fluorescent protein was inserted into  
10 the above pITL-1 polynucleotide vector forming the vector, pITL-1GFP. The  
computer generated sequence which approximates the sequence of pITL-1 GFP  
comprises:

15 GgtacctgccaccaTGGCGAAGGGCGAGGAACGTTCACTGGCGTGGTCCCAATT  
CTCGTGGAACTGGATGGCGATGTGAATGGCACAAATTCTGTCAAGCGG  
AGAGGGTGAAAGGTGATGCCACATACGGAAAGCTCACCTGAAATTATCT  
GCACCACTGGAAAGCTCCCTGTGCCATGGCCAACACTGGTCACTACCTTC  
ACCTATGGCGTGCAGTGCTTTCCAGATAACCAGACCATATGAAGCAGCA  
TGACTTTTCAAGAGCGCCATGCCGAGGGCTATGTGCAGGAGAGAACCA  
TCTTTTCAAAGATGACGGGAACtACAAGACCCCGCCTGAAGTCAAGTTC  
GAAGGTGACACCCCTGGTGAATAGAATCGAGTTGAAGGGCATTGACTTAA  
GGAAGATGGAAACATTCTCGGCCACAAGCTGGAATACAACtATAACTCCC  
ACAATGTGTACATCATGGCCGACAAGCAAAAGAATGGCATCAAGGTCAA  
CTTCAAGATCAGACACAAACATTGAGGATGGATCCGTGCAGCTGGCCGACC  
20 ATTATCAACAGAACACTCCAATCGCGACGGCCCTGTGCTCCTCCCAGAC  
AACCATACCTGTCCACCCAGTCTGCCCTGTCTAAAGATCCAACGAAAA  
GAGAGACCACATGGCCTGCTGGAGTTGTGACCGCTGCTGGGATCACAC  
ATGGCATGGACGAGCTGTACAAGTGAGCGCtttaagggccaTATGGTGAGTGG  
TGCCTGACCCAGGCAGGGATGGGGAGACCTGTAGTCAGAGCCCCGG  
30 GCAGCACAGGCCAATGCCGTCTCCCTGCAGGATgAGTagtgagtgccttcct  
gGCCtGGaaatggccactccatgtcccaccgttgtccataaaaattaaatgtgcatttttgtcactgt  
cctctataatattataagatgttatcGAATTCTTCGGACTTTGAAAGTGTGGTGG  
GGGAAGGATTCGAACCTTCGAAGTCGATGACGGCAGATTAGAGTCTGCT  
35 CCCTTGCCGCTGGAACCCCCACCACGGTAATGCTTTACTGGCCTG  
CTCCCTTATCGGGAAAGCGGGCGCATCATCAAATGACCGCCGCTGTA  
AAGTGTACGTTGAGAAAGAATTCCtgcagccccGCCGCGTTGCTGGCGTTTC  
CATAGGCTCCGCCCTGACGAGCATCACAAAATGACGCTCAAGTCA  
GAGGTGGCGAAACCCGACAGGACTATAAGATACCAGGCGTTCCCCCTG  
40 GAAGCTCCCTCGTGCCTCCTGTTCCGACCCCTGCCGTTACCGGATAC  
CTGTCCGCCCTTCTCCCTCGGAAGCGTGGCGCTTCTCAATGCTCACGC  
TGTAGGTATCTCAGTTGGTGTAGGTCGTTGCTCCAAGCTGGGCTGTGTG  
CACGAACCCCCCGTTCAAGCCGACCGCTGCGCCTTATCCGGTAACATATCG  
TCTTGAGTCCAACCCGGTAAGACACGACTTATGCCACTGGCAGCAGCCA  
CTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTC  
45 TTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTGGTAT

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5 CTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTT  
GATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTGTTGCAAG  
CAGCAGATTACGCCAGAAAAAAGGATCTggggatccggagagctccaaacgcgtgg  
atgcattggatgaggaaaggaggtaagatctgtaatgaataagcaggaacttgaagactcagtgactcagtgagtaataaa  
gactcagtgacttctgatccctgtctaactgccactccctgttgtcccaagaaaagcggctccctgtctctgaggaggaccctt  
ccctggaaaggtaaaaactaaggatgtcagcagagaaaattttccaccattggtgcttggtaaaagaggaaactgatGAGC  
10 TCACTCTAGATGAGAGAGCAGTGAGGGAGAGACAGAGACTCGAATTCC  
GGAGCTATTCACTTCTTTCCGTTTGCAATTCACTTATGATACC  
GGCCAATGCTTGGTTGCTATTTGGAAACTCCCCCTAGGGATGCCCTC  
AACTGGCCCTATAAAGGGCCAGCCTGAGCTGCAGAGGATTCTGCAGAGG  
ATCAAGACACGTGGACCTCGCACAGCCTCTCCCACA (SEQ ID NO. 29)

15 The pITL-1-GFP vector was tested for functional expression in primary human skeletal muscle cells. The vector pITL-GFP expressed the reporter protein, GFP in the human skeletal muscle cells in vitro.

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## **SEQUENCE LISTING**

(1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: THE GOVERNMENT OF THE UNITED  
STATES OF AMERICA AS REPRESENTED BY THE  
SECRETARY, DEPARTMENT OF HEALTH AND HUMAN  
SERVICES  
(B) STREET: OFFICE OF TECHNOLOGY TRANSFER,  
                  SUITE 325  
                  6011 EXECUTIVE BOULEVARD  
(C) CITY: ROCKVILLE  
(D) STATE OR PROVINCE: MARYLAND  
(E) COUNTRY: UNITED STATES OF AMERICA  
(F) POSTAL CODE: 20852

(i) APPLICANT:

- (A) NAME: NELSON, EDWARD L.  
(B) STREET: 660 BUCKHORN ROAD  
(C) CITY: ELDERSBURG  
(D) STATE OR PROVINCE: MARYLAND  
(E) COUNTRY: UNITED STATES OF AMERICA  
(F) POSTAL CODE: 21784

(i) APPLICANT:

- (A) NAME: NELSON, PETER J.  
(B) STREET: REUTTERSTRASSE 70  
(C) CITY: MUNICH  
(E) COUNTRY: GERMANY  
(F) POSTAL CODE: 80689

(ii) TITLE OF INVENTION: NOVEL VECTOR FOR  
POLYNUCLEOTIDE VACCINES

(iii) NUMBER OF SEQUENCES: 29

(iv) CORRESPONDENCE ADDRESS:

- (A) ADDRESSEE: MORGAN & FINNEGAN, L.L.P.  
(B) STREET: 345 PARK AVENUE  
(C) CITY: NEW YORK  
(D) STATE: NEW YORK  
(E) COUNTRY: USA  
(F) ZIP: 10154

(v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: DISKETTE, 3.5 INCH, 1.44 MB
  - (B) COMPUTER: IBM PC COMPATIBLE
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: WORDPERFECT 5.1

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- (vi) CURRENT APPLICATION DATA:
  - (A) FILING DATE: 14-AUG-1997
- (vii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: US60/023931
  - (B) FILING DATE: 14-AUG-1996
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: KATHRYN M. BROWN
  - (B) REGISTRATION NUMBER: 34556
  - (C) REFERENCE/DOCKET NUMBER: 2026-4236PC
- (ix) TELECOMMUNICATION INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 453 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:1:

GGCCGCGTTG	CTGGCGTTT	TCCATAGGCT	CCGGCCCCCT	40
GACGAGCATC	ACAAAAATCG	ACGCTCAAGT	CAGAGGTGGC	80
GAAACCCGAC	AGGACTATAA	AGATACCAGG	CGTTTCCCCC	120
TGGAAGCTCC	CTCGTGCCT	CTCCTGTTCC	GACCCTGCCG	160
CTTACCGGAT	ACCTCTCCGC	CTTTCTCCCT	TCGGGAAGCG	200
TGGCGTTTC	TCAATGCTCA	CGCIGTAGGT	ATCTCAGTT	240
GGTGTAGGTC	GTTCGCTCCA	AGCTGGGCTG	TGTGCACGAA	280
CCCCCGTIC	AGCCCGACCG	CTGCGCCTTA	TCCGGTAACT	320
ATCGTCTTGA	GTCCAACCCG	GTAAGACACG	ACTTATCGCC	360
ACTGGCAGCA	GCCACTGGTA	ACAGGATTAG	CAGAGCGAGG	400
TATGTAGGCG	GTGCTACAGA	GTTCTTGAAG	TGGTGGCCTA	440
ACTACGGCTA	CAC			453

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 453 base pairs
  - (B) TYPE: nucleic acid

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(C) STRANDEDNESS: single  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GTGTAGCCGT	AGTTAGGCCA	CCACTTCAAG	AACTCTGTAG	40
CACCGCCTAC	ATACCTCGCT	CTGCTAATCC	TGTTACCAGT	80
GGCTGCTGCC	AGTGGCGATA	AGTCGTGTCT	TACCGGGTTG	120
GACTCAAGAC	GATAGTTACC	GGATAAGGCG	CAGCGGTCGG	160
GCTGAACGGG	GGGTTCGTGC	ACACAGCCC	GCTTGGAGCG	200
AACGACCTAC	ACCGAACTGA	GATAACCTACA	CCGTGAGCAT	240
TGAGAAAGCG	CCACCGTTCC	CGAAGGGAGA	AAGGCGGACA	280
GGTATCCGGT	AAGCGGCAGG	GTCGGAACAG	GAGAGCGCAC	320
GAGGGAGCTT	CCAGGGGGAA	ACGCCCTGGTA	TCTTTATAGT	360
CCTGTGGGT	TTGCCACCT	CTGACTTGAG	CGTCGATT	400
TGTGATGCTC	GTCAGGGGGG	CGGAGCCTAT	GGAAAAACGC	440
CAGAACGCG	GCC			453

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 210 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAATTCTTC	GGACTTTGA	AAGTGATGGT	GGTGGCCGAA	40
GGATTCGAA	CTTCGAAGTC	GATGACGGCA	GATTTAGAGT	80
CTGCTCCCT	TGGCCGCTCG	GGAAACCCAC	CACGGGTAA	120
GCTTTACTG	GCCTGCTCCC	TTATCAGGAA	CGGGGGCGCA	160
TCATATCAA	TGACGCGCCG	CTGTAAAGTG	TTACGTTGAG	200
AAAGAATT	C			210

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 209 base pairs

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- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:4:

GAATTCTTTC	TCAACGTAAC	ACTTTACAGC	GGCGCGTCAT	40
TTGATATGAT	GCGCCCCGCT	TCCCGATAAG	GGAGCAGGCC	80
AGTAAAAGCA	TTACCCGTGG	TGGGGTTCGG	GAGCGGCCAA	120
AGGGAGCAGA	CTCTAAATCT	GCCGTCATCG	ACTTCGAAGG	160
TTCGAATCCT	TCCCCCACCA	CCATCACTTT	AAAAAGTCCG	200
AAAGAATTC				209

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 6 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:5:

AATAAA

6

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 6 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:6:

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ATTAAA

6

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 6 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:7:

AGTAAA

6

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 6 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:8:

AAGAAC

6

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 6 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No

- 80 -

(iv) ANTI-SENSE: No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AATACA

6

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 228 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GCCTTAAGGG	CCATATGGTG	AGTGGATCCC	TTGACCCCCAG	40
GCGGGGATGG	GGAGACCTG	TAGTCAGAGC	CCCCGGGGCAG	80
CACAGGCCAA	TGCCCGTCCT	TCCCCTGCAG	GATGAGTAGT	120
GAGTGCCTCT	CCTGGCCCTG	GAAGTTGCCA	CTCCAGTGCC	160
CACCAGCCTT	GTCCTAATAA	AATTAAGTTG	CATCATTTG	200
TCTGACTAGG	TGTCCCTCTAT	AATATTAT		228

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 228 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATAATATTAT	AGAGGACACC	TAGTCAGAAC	AAATGATGCA	40
ACTTAATTCTT	ATTAGGACAA	GGCTGGTGGG	CACTGGAGTG	80
GCAACTTCCA	GGGCCAGGAG	AGGCACTCAC	TACTCATCCT	120

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GCAGGGGAAG GACGGGCATT GGCCTGTGCT GCCCGGGGGC	160
TCTGACTACA GGTCTCCCCC ATCCCCGCCT GGGGTCAAGG	200
CATCCACTCA CCATATGGCC CTTAAGG	228

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 252 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:12:

CCTCGGTACC TGCCATGGCG CGGATTCTTT ATCACTGATA	40
AGTTGGTCCA CATATTATGT TTATCAGTGA TAAAGTGTCA	80
AGCATGACAA AGTTCAGGCC GAATACAGTG ATCCGTGCCG	120
GCCCTGGACT GTTGAACGAG GTCGGCGTAG ACGGTCTGAC	160
GACACGCAAA CTGGCGAAC GGTTGGGGGT GCAGCAGCCG	200
GCGCTTTACT GGCACATTCAAG GAACAAGCGG GCGCCTTAAG	240
GGCCATATGC CG	252

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 35 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:13:

CCTCGGTACC TGCCACCATG GCGCGGATTC TTTAT	35
--	----

(2) INFORMATION FOR SEQ ID NO:14:

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- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 38 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:14:

CGGCATATGG CCTTAAGGCG CCCGCTTGTT CCTGAAGT

38

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 228 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:15:

GCCTTAAGGG	CCATATGGTG	AGTGGATGCC	TTGACCCCAG	40
GCAGGGATGG	GGGAGACCTG	TAGTCAGAGC	CCCCGGGCAG	80
CACAGGCCAA	TGCCCCCTCCT	TCCCCCTGCAG	GATGAGTAGT	120
GAGTGCCTCT	CCTGGCCCTG	GAAGTTGCCA	CTCCAGTGCC	160
CACCAGCCTT	GTCCTAATAA	AATTAAGTTG	CATCATTTTG	200
TCTGACTAGG	TGTCCCTCAT	AATATTAT		228

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1425 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA

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(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TGCCATGGCG	CGGATTCTTT	ATCACTGATA	AGTTGGTGGA	40
CATATTATGT	TTATCAGTGA	TAAAGTGTCA	AGCATGACAA	80
AGTTGCAGCC	GAATACAGTG	ATCCGTGCCG	GCCCTGGACT	120
GTTGAACGAG	GTCGGCGTAG	ACGGTCTGAC	GACACGCAA	160
CTGGCGGAAC	GGTTGGGGGT	GCAGCAGCCG	GCGCTTTACT	200
GGCACTTCAG	GAACAAGCGG	GCGCCTTAAG	GGCCATATGG	240
TGAGTGGATG	CCTTGACCCC	AGGCGGGGAT	GGGGGAGACC	280
TGTAGTCAGA	GCCCCCGGGC	AGCACAGGCC	AATGCCCGTC	320
CTTCCCCCTGC	AGTGAGTAGT	GAUTGCCCGG	GTGGGATCCC	360
TGTGACCCCT	CCCCAGTGCC	TCTCCTGGCC	CTGGAAGTTG	400
CCACTCCAGT	GCCCCACCAGC	CTTGTCTCAA	TAAAATTAAG	440
TTGCATCATT	TTGTCTGACT	AGGTGTCTC	TATAATATTA	480
TAAGCTTGAT	ATCGAATTCT	TTCTCAACGT	AACACTTTAC	520
AGCGGCGCGT	CATTGATAT	GATGCGCCCC	GCTTCCCGAT	560
AAGGGAGCAG	GCCAGTAAAA	GCATTACCCG	TGGTGGGGTT	600
CCCGAGCGGC	CAAAGGGAGC	AGACTCTAAA	TCTGCCGTCA	640
TCGACTTCGA	AGGTTCGAAT	CCTTCCCCCA	CCACCATCAC	680
TTTCAAAAGT	CCGAAAGAAT	TCCTGCAGCC	CGTGTAGCCG	720
TAGTTAGGCC	ACCACTCAA	GAACCTCTGA	GCACCGCCTA	760
CATAACCTCGC	TCTGCTAATC	CTGTTACCAAG	TGGCTGCTGC	800
CAGTGGCGAT	AAGTCGTGTC	TTACCGGGTT	GGACTCAAGA	840
CGATAGTTAC	CGGATAAGGC	GCAGCGGTG	GGCTGAACGG	880
GGGGTTCGTG	CACACAGCCC	AGCTTGGAGC	GAACGACCTA	920
CACCGAACTG	AGATAACCTAC	AGCGTGAGCA	TTGAGAAAGC	960
GCCACGCTTC	CCGAAGGGAG	AAAGGGCGAC	AGGTATCCGG	1000
TAAGCGGCAG	GGTCGGAACA	GGAGAGCGCA	CGAGGGAGCT	1040
TCCAGGGGGA	AACGCCCTGGT	ATCTTTATAG	TCCTGTCGGG	1080
TTTCGCCACC	TCTGACTTGA	GCCTCGATT	TTGTGATGCT	1120
CGTCAGGGGG	GCCTGAGCTA	TGGAAAAACG	CCAGCAACGC	1160
GGCCGGGGGA	TCCGGAGAGC	TCACTCTAGA	TGAGAGAGCA	1200
GTGAGGGAGA	GACAGAGACT	CGAATTTCG	GAGCTATTTC	1240
AGTTTTCTT	TCCGTTTTGT	GCAATTTCAC	TTATGATACC	1280
GGCCAATGCT	TGGTTGCTAT	TTTGGAAACT	CCCCTTAGGG	1320
GATGCCCTC	AACTGGCCCT	ATAAAGGGCC	AGCCTGAGCT	1360
GCAGAGGATT	CCTGCAGAGG	ATCAAGACAG	CACGTGGACC	1400
TCGCACAGCC	TCTCCCACAG	GTACC		1425

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 720 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown

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- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:17:

ATGAGCAAG	GGCGAGGAAC	TGTTCACTGG	CGTGGTCCA	40
ATTCTCGTGG	AACTGGATGG	CGATGTGAAT	GGGCACAAAT	80
TTTCTGTCAG	CGGAGAGGGT	GAAGGTGATG	CCACATACGG	120
AAAGCTCACC	CTGAAATTCA	TCTGCACCAAC	TGGAAAGCTC	160
CCTGTGCCAT	GGCCAACACT	GGTCACTAACC	TTCACCTATG	200
GCGTGCAGTG	CTTTCCAGA	TACCCAGACC	ATATGAACGA	240
GCATGACTTT	TTCAAGAGCG	CCATGCCCGA	GGGCTATGTG	280
CAGGAGAGAA	CCATCTTTT	CAAAGATGAC	GGGAACTACA	320
AGACCCGCGC	TGAAGTCAAG	TTCGAAGGTG	ACACCCCTGGT	360
GAATAGAACATC	GAGTTGAAGG	GCATTGACTT	TAAGGAAAGAT	400
GGAAACATTC	TCGGGCCACAA	GCTGGAATAC	AACTATAACT	440
CCCACAATGT	GTACATCATG	GCCGACAAGC	AAAAGAATGG	480
CATCAAGGTC	AACTCAAGA	TCAGACACAA	CATTGAGGGAT	520
GGATCCGTGC	AGCTGGCCGA	CCATTATCAA	CAGAACACTC	560
CAATCGGCAG	CCGCCCTGTG	CTCCTCCCAG	ACAACAATTA	600
CCTGTCCACC	CAGTCTGCC	TGTCTAAAGA	TCCCAACGAA	640
AAGAGAGAGACC	ACATGGTCCT	GCTGGAGTTT	GTGACCGCTG	680
CTGGGATCAC	ACATGGCATG	GACGAGCTGT	ACAAGTGAGC	720

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1911 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:18:

TATGAGCAAG	GGCGAGGAAC	TGTTCACTGG	CGTGGTCCA	40
ATTCTCGTGG	AACTGGATGG	CGATGTGAAT	GGGCACAAAT	80
TTTCTGTCAG	CGGAGAGGGT	GAAGGTGATG	CCACATACGG	120
AAAGCTCACC	CTGAAATTCA	TCTGCACCAAC	TGGAAAGCTC	160
CCTGTGCCAT	GGCCAACACT	GGTCACTAACC	TTCACCTATG	200
GCGTGCAGTG	CTTTCCAGA	TACCCAGACC	ATATGAAGCA	240
GCATGACTTT	TTCAAGAGCG	CCATGCCCGA	GGGCTATGTG	280

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CAGGAGAGAA	CCATCTTTT	CAAAGATGAC	GGGAACTACA	320
AGACCCCGCGC	TGAAGTCAAG	TTCGAAGGTG	ACACCCCTGGT	360
GAATAGAACATC	GAGTTGAAGG	GCATTGACTT	TAAGGAAGAT	400
GGAAACATTC	TCGGCCACAA	GCTGGAATAC	AACTATAACT	440
CCCACAATGT	GTACATCATG	GCCGACAAGC	AAAAGAATGG	480
CATCAAGGTC	AACTTCAAGA	TCAGACACAA	CATTGAGGAT	520
GGATCCGTGC	AGCTGGCCGA	CCATTATCAA	CAGAACACTC	560
CAATCGGCCA	CGGCCCTGTG	CTCCTCCCAG	ACAACCATTA	600
CCTGTCCACC	CAGTCTGCC	GTCTAAAGAT	CCCAACGAAA	640
AGAGAGACCA	CATGGTCCTG	CTGGAGTTG	TGACCGCTGC	680
TGGGATCACA	CATGGCATGG	ACGAGCTGTA	CAAGTGAAGCC	720
ATATGGTGAG	TGGATGCCTT	GACCCCAGGC	GGGGATGGGG	760
GAGACCTGTA	GTCAGAGCCC	CCGGGCAGCA	CAGGCCAATG	800
CCCGTCCTTC	CCCTGCAGTG	AGTAGTGACT	GCCCCGGTGG	840
GATCCCTGTG	ACCCCTCCCC	AGTGCCTCTC	CTGGCCCTGG	880
AAGTTGCCAC	TCCAGTCCCC	ACCAGCCTTG	TCCTAATAAA	920
ATTAAGTTGC	ATCATTGTTG	CTGACTAGGT	GTCCTCTATA	960
ATATTATAAG	CTTGATATCG	AATTCTTCT	CAACGTAACA	1000
CTTTACAGCG	GCGCGTCATT	TGATATGATG	CGCCCCGCTT	1040
CCCGATAAAGG	GAGCAGGCCA	GTAAAAGCAT	TACCCGTGGT	1080
GGGGTTCCCG	AGCGGCCAAA	GGGAGCAGAC	TCTAAATCTG	1120
CCGTACATCGA	CTTCGAAGGT	TCGAATCCTT	CCCCCACAC	1160
CATCACTTTC	AAAAGTCCGA	AAGAATTCTT	GCAGCCCCTG	1200
TAGCCGTAGT	TAGGCCACCA	CTTCAAGAAC	TCTGTAGCAC	1240
CGCCTACATA	CCTCGCTCTG	CTAATCCTGT	TACCAGTGGC	1280
TGCTGCCAGT	GGCGATAAGT	CGTGTCTTAC	CGGGTTGGAC	1320
TCAAGACGAT	AGTTACCGGA	TAAGGCGCAG	CGGTCCGGCT	1360
GAACGGGGGG	TTCGTGCACA	CAGCCCAGCT	TGGAGCGAAC	1400
GACCTACACC	GAACTGAGAT	ACCTACAGCG	TGAGCATTGA	1440
GAAAGCGCCA	CGCTTCCCAG	AGGGAGAAAG	GC GGACAGGT	1480
ATCCGGTAAG	CGGCAGGGTC	GGAACAGGAG	AGCGCACGAG	1520
GGAGCTTCCA	GGGGGAAACG	CCTGGTATCT	TTATAGTCCT	1560
GTCGGGTTTC	GCCACCTCTG	ACTTGAGCGT	CGATTTTGT	1600
GATGCTCGTC	AGGGGGCGG	AGCCTATGGA	AAAACGCCAG	1640
CAACCGGCC	GGGGGATCCG	GAGAGCTCAC	TCTAGATGAG	1680
AGAGCAGTGA	GGGAGAGACA	GAGACTCGAA	TTTCCGGAGC	1720
TATTCAGTT	TTCTTTCCG	TTTTGTGCAA	TTTCACCTTAT	1760
GATACCGGCC	AATGCTTGGT	TGCTATTTG	GAAACTCCCC	1800
TTAGGGGATG	CCCCTCAACT	GGCCCTATAA	AGGGCCAGCC	1840
TGAGCTGCAG	AGGATTCCTG	CAGAGGATCA	AGACAGCAGC	1880
TGGACCTCGC	ACAGCCTCTC	CCACAGGTAC	C	1911

## (2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 69 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:19:

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Pro Asp Leu Ser Tyr Met Pro Ile Trp Lys Phe Pro  
1 5 10  
Asp Glu Glu Gly Ala Cys Gln Pro Cys Pro Ile Asn  
15 20  
Cys Thr His Ser Cys Val Asp Leu Asp Asp Lys Gly  
25 30 35  
Cys Pro Ala Glu Gln Arg Ala Ser Pro Leu Thr Ser  
40 45  
Ile Ile Ser Ala Val Val Gly Ile Leu Leu Val Val  
50 55 60  
Val Leu Gly Val Val Phe Gly Ile Leu  
65

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 287 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:20:

Pro Ala Pro Gly Ala Gly Gly Met Val His His Arg  
1 5 10  
His Arg Ser Ser Ser Thr Arg Ser Gly Gly Gly Asp  
15 20  
Leu Thr Leu Gly Leu Glu Pro Ser Glu Glu Ala  
25 30 35  
Pro Arg Ser Pro Leu Ala Pro Ser Glu Gly Ala Gly  
40 45  
Ser Asp Val Phe Asp Gly Asp Leu Gly Met Gly Ala  
50 55 60  
Ala Lys Gly Leu Ser Leu Pro Thr His Asp Pro Ser  
65 70  
Pro Leu Gln Arg Tyr Ser Glu Asp Pro Thr Val Pro  
75 80  
Leu Pro Ser Glu Thr Asp Gly Tyr Val Ala Pro Leu  
85 90 95  
Thr Cys Ser Pro Gln Pro Glu Tyr Val Asn Gln Pro  
100 105  
Asp Val Arg Pro Pro Pro Ser Pro Arg Glu Gly Pro  
110 115 120  
Leu Pro Ala Ala Arg Pro Ala Gly Ala Thr Leu Glu  
125 130  
Arg Pro Lys Thr Leu Ser Pro Gly Lys Asn Gly Val  
135 140

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Val Lys Asp Val Phe Ala Phe Gly Gly Ala Val Glu  
145 150 155  
Asn Pro Glu Tyr Leu Thr Pro Gln Gly Thr Cys Ser  
160 165  
Pro Gln Pro Glu Tyr Val Asn Gln Pro Asp Val Arg  
170 175 180  
Pro Gln Pro Pro Ser Pro Arg Glu Gly Pro Leu Pro  
185 190  
Ala Ala Arg Pro Ala Gly Ala Thr Leu Glu Arg Pro  
195 200  
Lys Leu Ser Pro Gly Lys Asn Gly Val Val Lys Asp  
205 210 215  
Val Phe Ala Phe Gly Gly Ala Val Glu Asn Pro Glu  
220 225  
Tyr Leu Thr Pro Gln Gly Gly Ala Ala Pro Gln Pro  
230 235 240  
His Pro Pro Pro Ala Phe Ser Pro Ala Phe Asp Asn  
245 250  
Leu Tyr Tyr Trp Asp Asp Pro Pro Glu Arg Gly Ala  
255 260  
Pro Pro Ser Thr Phe Lys Gly Thr Pro Thr Ala Glu  
260 270 275  
Asn Pro Glu Tyr Leu Gly Leu Asp Val Pro Val  
280 285

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 22 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:21:

Ile Ile Ser Ala Val Val Gly Ile Leu Leu Val Val  
1 5 10  
Val Leu Gly Val Val Phe Gly Ile Leu Ile  
15 20

(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 2145 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: unknown

- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

GCCACCATGG	CCCCTGACCT	CTCCTACATG	CCCATCTGGA	40
AGTTTCCAGA	TGAGGAGGGC	GCATGCCAGC	CTTGCCCCAT	80
CAACTGCACC	CACTCCTGTG	TGGACCTGGA	TGACAAGGGC	120
TGCCCGGCCG	AGCAGAGAGC	CAGCCCTCTG	ACGTCCATCA	160
TCTCTGCGGT	GGTTGGCATT	CTGCTGGTCG	TGGTCTTGGG	200
GGTGGTCTTT	GGGATCCTCA	TCAAGCGACG	GCAGCAGAAAG	240
ATCACATGTC	CAGACCTGTC	CCCGGGCGCT	GGGGGCATGG	280
TCCACCACAG	GCACCGCAGC	TCATCTACCA	GGAGTGGCGG	320
TGGGGACCTG	ACACTAGGGC	TGGAGCCCTC	TGAAGAGGAG	360
GCCCCCAGGT	CTCCACTGGC	ACCCCTCCGAA	GGGGCTGGCT	400
CCGATGTATT	TGATGGTGAC	CTGGGAATGG	GGGCAGCCAA	440
GGGGCTGCAA	AGCCTCCCCA	CACATGACCC	CAGCCCTCTA	480
CAGCGGTACA	GTGAGGACCC	CACAGTACCC	CTGCCCTCTG	520
AGACTGATGG	CTACGTTGCC	CCCCTGACCT	GCAGCCCCCA	560
GCCTGAATAT	GTGAACCAGC	CAGATGTTCG	GCCCCAGCCC	600
CCTTCGCCCC	GAGAGGGCCC	TCTGCCTGCT	GCCCCGACCTG	640
CTGGTGCCAC	TCTGGAAAGG	CCCAAGACTC	TCTCCCCAGG	680
GAAGAATGGG	GTCGTCAAAG	ACGTTTTGC	CTTTGGGGGT	720
GCCGTGGAGA	ACCCCAGAC	TTGACACCCC	AGGGAGGAGC	760
TGCCCTCTAG	CCCCACCCCTC	CTCCTGCCTT	CAGCCCAGCC	800
TTCGACAAACC	TCTATTACTG	GGACCAGGAC	CCACCAGAGC	840
GGGGGGCTCC	ACCCAGCACC	TTCAAAGGGA	CACCTACGGC	880
AGAGAACCCA	GAGTACCTGG	GTCTGGACGT	GCCAGTGTGA	920
AGCCTTAAGG	GCCATATGGT	GAGTGGATGC	CTTGACCCCA	960
GGCGGGGATG	GGGGAGACCT	GTAGTCAGAG	CCCCCGGGCA	1000
GCACAGGCCA	ATGCCCGTCC	TTCCCCTGCA	GTGAGTAGTG	1040
ACTGCCCCGG	TGGGATCCCT	GTGACCCCTC	CCCAGTGCT	1080
CTCCTGGCCC	TGGAAGTTGC	CACTCCAGTG	CCCACCAAGCC	1120
TTGTCTTAAT	AAAATTAAGT	TGCATCATTT	TGTCTGACTA	1160
GGTGTCTCT	ATAATATTAT	AAGCTTGATA	TCGAATTCTT	1200
TCTCAACGTA	ACACTTTACA	CGGGCGCGTC	ATTTGATATG	1240
ATGCGCCCCG	CTTCCCATA	AGGGAGCAGG	CCAGTAAAAG	1280
CATTACCGT	GGTGGGGTTC	CCGAGCGGCC	AAAGGGAGCA	1320
GACTCTAAAT	CTGCCGTCAT	CGACTTCGAA	GGTTCGAATC	1360
CTTCCCCCAC	CACCATCACT	TTCAAAAGTC	CGAAAGAATT	1400
CCTGCAGCCC	GTGTAGCCGT	AGTTAGGCCA	CCACTTCAAG	1440
AACTCTGTAG	CACCGCCTAC	ATACCTCGCT	CTGCTAATCC	1480
TGTTACCAGT	GGCTGCTGCC	AGTGGCGATA	AGTCGTGTCT	1520
TACCGGGTTG	GACTCAAGAC	GATAGTTACC	GGATAAGGCG	1560
CAGCGGTCGG	GCTGAACGGG	GGGTTCGTGC	ACACAGCCCA	1600
GCTTGGAGCG	AACGACCTAC	ACCGAACTGA	GATACCTACA	1640
GCGTGAGCAT	TGAGAAAGCG	CCACGCTTCC	CGAAGGGAGA	1680
AAGGCGGACA	GGTATCCGGT	AAGCGGCAGG	GTCGGAACAG	1720
GAGAGCGCAC	GAGGGAGCTT	CCAGGGGAA	ACGCCTGGTA	1760

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TCTTTATAGT CCTGTCGGGT TTGCCACCT CTGACTTGAG	1820
CGTCGATTG TGTGATGCTC GTCAGGGGG CCGAGCCTAT	1860
GGAAAAACGC CAGCAACGCG GCCGGGGAT CCGGAGAGCT	1900
CACTCTAGAT GAGAGAGCAG TGAGGGAGAG ACAGAGACTC	1940
GAATTTCCGG AGCTATTCA GTTTCTTT CCGTTTGTG	1980
CAATTTCACT TATGATAACCG GCCAATGCTT GGTTGCTATT	2020
TTGGAAACTC CCCCTAGGGG ATGCCCTCA ACTGGCCCTA	2060
TAAAGGGCCA GCCTGAGCTG CAGAGGATTC CTGCAGAGGA	2100
TCAAGACAGC ACGTGGACCT CGCACAGCCT CTCCCACAGG	2140
TACCT	2145

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 27 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: oligonucleotide
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:23:

GTCTGCCACC ATGGCCTACT CCCCTGC

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(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 36 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: oligonucleotide
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:24:

TTCTTTGGTG ACCTACCTCT TCGGAATTGC CGAGTC

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(2) INFORMATION FOR SEQ ID NO:25:

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- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1242 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

ATGGAGGAGC	CGCAGTCAGA	TCCTAGCGTC	GAGCCCCCTC	40
TGAGTCAGGA	AACATTTCAGA	GACCTATGGA	AACTACTTCC	80
TGAAAACAAC	GTTCTGTCCC	CCTTGCCGTC	CCAAGCAATG	120
GATGATTGAA	TGCTGTCCCC	GGACGATATT	GAACAATGGT	160
TCACTGAAGA	CCCAGGTCCA	GATGAAGCTC	CCAGAACGCC	200
AGAGGCTGCT	CCCCGCCTGG	CCCCTGCACC	AGCAGCTCCT	240
ACACCCGGCGG	CCCCTGCACC	AGCCCCCTCC	TGGCCCCCTGT	280
CATCTTCTGT	CCCTTCCCAG	AAAACCTACC	AGGGCAGCTA	320
CGGTTTCCGT	CTGGGCTTCT	TGCATTCTGG	GACAGCCAAG	360
TCTGCCACCA	TGGCCTACTC	CCCTGCCTCT	GTGACTTGCA	400
CGTACTCCCC	TGCCCTCAAC	AAGATGTTT	GCCAACCTGGC	440
CAAGACCTGC	CCTGTGCAGC	TGTGGGTTGA	TTCCACACCC	480
CCGCCCGGCA	CCCGCGTCCG	CGCCATGGCC	ATCTACAAGC	520
AGTCACAGCA	CATGACGGAG	GTTGTGAGGC	GCTGCCCTCA	560
CCATGAGCGC	TGCTCAGATA	GCGATGGTCT	GGCCCCCTCCT	600
CAGCGTCTTA	TCCGAGTGGA	AGGAAATTG	CGTGTGGAGT	640
ATTTGGATGA	CAGAAAACACT	TTTCGACATA	GTGTGGTGGT	680
GCCCTATGAG	CCGCCTGAGG	TTGGCTCTGA	CTGTACCCACC	720
ATCCACTACA	ACTACATGTG	TAACAGTTCC	TGCATGGCG	760
GCATGAACCG	GAGGCCCATC	CTCACCATCA	TCACACTGGA	800
AGACTCCAGT	GGTAATCTAC	TGGGACGGAA	CAGCTTGAG	840
GTGCGTGTCT	GTGCCGTCTCC	TGGGAGAGAC	CGGCGCACAG	880
AGGAAGAGAA	TCTCCGCAAG	AAAGGGGAGC	CTCACCCACGA	920
GCTGCCCTCA	GGGAGCACTA	AGCGAGCACT	GCCCAACAAC	960
ACCAGCTCCT	CTCCCCAGCC	AAAGAAGAAA	CCACTGGATG	1000
GAGAATATTT	CACCCCTTCAG	ATCCGTGGGC	GTGAGCGCTT	1040
CGAGATGTTC	TTTGGTGACC	TACCTCTTCG	GAATTGCCGA	1080
GTCTTCCGAG	AGCTGAATGA	GGCCTTGAA	CTCAAGGATG	1120
CCCAGGCTGG	GAAGGAGCCA	GGGGGGAGCA	GGGCTCACTC	1160
CAGCCACCTG	AAGTCCAAA	AGGGTCAGTC	TACCTCCCGC	1200
CATAAAAAAC	TCATGTTCAA	GACAGAAGGG	CCTGACTCAG	1240
AC				1242

- (2) INFORMATION FOR SEQ ID NO:26:

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- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 608 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:26:

CTCGGGCCGC	GTTGCTGGCG	TTTTTCCATA	GGCTCCGCC	40
CCCTGACGAG	CATCACAAAA	ATCGACGCTC	AAGTCAGAGG	80
TGGCGAAACC	CGACAGGACT	ATAAAAGATAC	CAGGCGTTTC	120
CCCCTGGAAG	CTCCCTCGTG	CGCTCTCCTG	TTCCGACCC	160
GCCGCTTACC	GGATAACCTGT	CCGCCTTTCT	CCCTTCGGGA	200
AGCGTGGCGC	TTCTCAATG	CTCACGCTGT	AGGTATCTCA	240
GTTCGGTGT	GGTCGTTCGC	TCCAAGCTGG	GCTGTGTGCA	280
CGAACCCCCC	GTTCAGCCCG	ACCGCTGCGC	CTTATCCGGT	320
AACTATCGTC	TGAGTCCAA	CCCGGTAAGA	CACGACTTAT	360
CGCCACTGGC	AGCAGCCACT	GGTAACAGGA	TTAGCAGAGC	400
GAGGTATGTA	GGCGGTGCTA	CAGAGTTCTT	GAAGTGGTGG	440
CCTAACTACG	GCTACACTAG	AAGGACAGTA	TTTGGTATCT	480
GCGCTCTGCT	GAAGCCAGTT	ACCTTCGGAA	AAAGAGTTGG	520
TAGCTCTTGA	TCCGGCAAAC	AAACCACCGC	TGGTAGCGGT	560
GGTTTTTTTG	TTTGCAGCA	GCAGATTACG	CGCAGAAAAAA	600
AAGGATCT				608

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1547 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:27:

GGTACCTGCC	ACCATGGCGC	GGATTCTTTA	TCACTGATAA	40
GTTGGTGGAC	ATATTATGTT	TATCAGTGAT	AAAGTGTCAA	80
GCATGACAAA	GTTGCAGCCG	AATACAGTGA	TCCGTGCCGG	120

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CCCTGGACTG	TTGAACGAGG	TCGGCGTAGA	CGGTCTGACG	160
ACACGCAAAC	TGGCGGAACG	GTTGGGGGTG	CAGCAGCCGG	200
CGCTTTACTG	GCACTTCAAGG	AACAAGCGGG	CGCCTTAAGG	240
GCCATATGGT	GAGTGGATGC	CTTGACCCC	GGCGGGGATG	280
GGGGAGACCT	GTAGTCAGAG	CCCCCGGGCA	GCACAGGCCA	320
ATGCCCGTCC	TTCCCCTGCA	GGATGAGTAG	TGAGTGCCTC	360
TCCTGGCCCT	GBAAGTTGCC	ACTCCAGTGC	CCACCAGCCT	400
TGTCCTAATA	AAATTAAAGTT	GCATCATTTC	GTCTGACTAG	440
GTGTCCCTCA	TAATATTATA	AGCTTGATAT	CGAATTCTT	480
CGGACTTTG	AAAGTGATGG	TGGTGGGGGA	AGGATTGAA	520
CCTTCGAAGT	CGATGACGGC	AGATTTAGAG	TCTGCTCCCT	560
TTGGCCGCTC	GGGAACCCC	CCACGGGTAA	TGCTTTTACT	600
GGCCTGCTCC	CTTATCGGGA	AGCGGGGCGC	ATCATATCAA	640
ATGACGCGCC	GCTGTAAAAGT	GTTACGTTGA	GAAAGAACATC	680
CTGCAGCCCG	CCGCGTTGCT	GGCGTTTTT	CATAGGCTCC	720
GCCCCCCTGA	CGAGCATCAC	AAAAATCGAC	GCTCAAGTCA	760
GAGGTGGCGA	AACCCGACAG	GACTATAAG	ATACCAGGCG	800
TTTCCCCCTG	GAAGCTCCCT	CGTGCCTCT	CCTGTTCCGA	840
CCCTGCCGCT	TACCGGATAC	CTGTCCGCCT	TTCTCCCTTC	880
GGGAAGCGTG	GCGCTTTCTC	AATGCTCACG	CTGTAGGTAT	920
CTCAGTTCGG	TGTAGGTCTG	TCGCTCCAAG	CTGGGCTGTG	960
TGCACGAACC	CCCCGTTCAAG	CCCGACCGCT	GCGCCTTATC	1000
CGGTAACTAT	CGTCTTGAGT	CCAACCCGGT	AAGACACGAC	1040
TTATCGCCAC	TGGCAGCAGC	CACTGGTAAC	AGGATTAGCA	1080
GAGCGAGGTA	TGTAGGCAGT	GCTACAGAGT	TCTTGAAGTG	1120
GTGGCCTAAC	TACGGCTACA	CTAGAAGGAC	AGTATTTGGT	1160
ATCTGCGCTC	TGCTGAAGCC	AGTTACCTTC	GGAAAAAGAG	1200
TTGGTAGCTC	TTGATCCGGC	AAACAAACCA	CCGCTGGTAG	1240
CGGTGGTTTT	TTTGTGGCA	AGCAGCAGAT	TACGCGCAGA	1280
AAAAAAGGAT	CTGGGGGATC	CGGAGAGCTC	ACTCTAGATG	1320
AGAGAGCACT	GAGGGAGAGA	CAGAGACTCG	AATTCCGGA	1360
GCTATTTCA	TTTTCTTTTC	CGTTTGTGC	AATTTCACCT	1400
ATGATAACGG	CCAATGCTTG	GTTGCTATT	TGGAAACTCC	1440
CCTTAGGGGA	TGCCCTCAA	CTGGCCCTAT	AAAGGGCCAG	1480
CCTGAGCTGC	AGAGGATTCC	TGCAGAGGAT	CAAGACAGCA	1520
CGTGGACCTC	GCACAGCCTC	TCCCACAA		1547

## (2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1847 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:28:

GGTACCTGCC	ACCATGGCGC	GGATTCTTTA	TCACTGATAA	40
GTGGTGGAC	ATATTATGTT	TATCAGTGAT	AAAGTGTCAA	80
GCATGACAAA	GTTGCAGCCG	AATACAGTGA	TCCGTGCCGG	120
CCCTGGACTG	TTGAACGAGG	TCGGCGTAGA	CGGTCTGACG	160
ACACGCAAAC	TGGCGGAACG	GTTGGGGTG	CAGCAGCCGG	200
CGCTTACTG	GCACTTCAAGG	AACAAGCGGG	CGCCTTAAGG	240
GCCATATGGT	GAGTGGATGC	CTTGACCCCCA	GGCGGGGATG	280
GGGGAGACCT	GTAGTCAGAG	CCCCCGGGCA	GCACAGGCCA	320
ATGCCCGTCC	TTCCCCTGCA	GGATGAGTAG	TGAGTGCCTC	360
TCCTGGCCCT	GGAAGTTGCC	ACTCCACTGC	CCACCCAGCCT	400
TGTCCTAATA	AAATTAAGTT	GCATCATTTC	GTCTGACTAG	440
GTGTCCCTCA	TAATATTATA	AGCTTGATAT	CGAATTCTTT	480
CGGACTTTG	AAAGTGTGG	TGGTGGGGGA	AGGATTGCAA	520
CCTTCGAAGT	CGATGACGGC	AGATTAGAG	TCTGCTCCCT	560
TTGGCCGCTC	GGGAACCCCCA	CCACGGGTAA	TGCTTTTACT	600
GGCCTGCTCC	CTTATCGGGA	AGCGGGCGC	ATCATATCAA	640
ATGACGCGCC	GCTGTAAAGT	GTTACGTTGA	GAAAGAATTC	680
CTGCAGCCCC	CCGCCTTGCT	GGCGTTTTTC	CATAGGCTCC	720
GCCCCCCTGA	CGAGCATCAC	AAAAATCGAC	GCTCAAGTCA	760
GAGGTGGCGA	AACCCGACAG	GAECTATAAAG	ATACCAGGCG	800
TTTCCCCCTG	GAAGCTCCCT	CGTGCGCTCT	CCTGTTCCGA	840
CCCTGCCGCT	TACCGGATAC	CTGTCCGCCT	TTCTCCCTTC	880
GGGAAGCGTG	GCGCTTTCTC	AATGCTCACG	CTGTAGGTAT	920
CTCAGTTCGG	TGTAGGTCGT	TCGCTCCAAG	CTGGGCTGTG	960
TGCACGAACC	CCCCGTTCAAG	CCCGACCGCT	GCGCCTTATC	1000
CGGTAACTAT	CGTCTTGAGT	CCAACCCGGT	AAGACACGAC	1040
TTATGCCAC	TGGCAGCAGC	CACTGGTAAC	AGGATTAGCA	1080
GAGCGAGGTA	TGTAGGCGGT	GCTACAGAGT	TCTTGAAGTG	1120
GTGGCCTAAC	TACGGCTACA	CTAGAAGGAC	AGTATTGGT	1160
ATCTGCGCTC	TGCTGAAGCC	AGTTACCTTC	GGAAAAAGAG	1200
TTGGTAGCTC	TTGATCCGGC	AAACAAAACCA	CCGCTGTTAG	1240
CGGTGGTTTT	TTTGTGTCAG	AGCAGCAGAT	TACGCGCAGA	1280
AAAAAAGGAT	CTGGGGGATC	CGGAGAGCTC	CCAACCGCGTT	1320
GGATGCATGG	ATGAGGGAAA	GGAGGTAAGA	TCTGTAATGA	1400
ATAAGCAGGA	ACTTTGAAGA	CTCAGTGACT	CAGTGAGTAA	1440
TAAAGACTCA	GTGACTTCTG	ATCCTGTCCT	AACTGCCACT	1480
CCTTGTGTC	CCAAGAAAGC	GGCTTCCTGC	TCTCTGAGGA	1520
GGACCCCTTC	CCTGGAAGGT	AAAACTAAGG	ATGTCAGCAG	1560
AGAAATTTTT	CCACCATTGG	TGCTTGGTCA	AAGAGGAAAC	1600
TGATGAGCTC	ACTCTAGATG	AGAGAGCAGT	GAGGGAGAGA	1640
CAGAGACTCG	AATTTCGGA	GCTATTTCA	TTTTCTTTTC	1680
CGTTTTGTGC	AATTTCACCTT	ATGATACCGG	CCAATGCTTG	1720
GTTGCTATTT	TGGAAACTCC	CCTTAGGGGA	TGCCCCCTCAA	1760
CTGGCCCTAT	AAAGGGCCAG	CCTGAGCTGC	AGAGGATTCC	1800
TGCAGAGGAT	CAAGACAGCA	CGTGGACCTC	GCACAGCCTC	1840
TCCCCACA				1847

## (2) INFORMATION FOR SEQ ID NO:29:

## (i) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 2308 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: unknown

- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GGTACCTGCC	ACCATGGCGA	AGGGCGAGGA	ACTGTTCACT	40
GGCGTGGTCC	CAATTCTCGT	GGAACTGGAT	GGCGATGTGA	80
ATGGGCACAA	ATTTTCTGTC	AGCGGGAGAGG	GTGAAGGTGA	120
TGCCACATAAC	GGAAAGCTCA	CCCTGAAATT	CATCTGCACC	160
ACTGGAAAGC	TCCCTGTGCC	ATGGCCAACA	CTGGTCACTA	200
CCTTCACCTA	TGGCGTGCAG	TGCTTTCCA	GATACCCAGA	240
CCATATGAAG	CAGCATGACT	TTTTCAAGAG	CGCCATGCC	280
GAGGGCTATG	TGCAGGAGAG	AACCATCTT	TTCAAAGATG	320
ACGGGAACTA	CAAGACCCGC	GCTGAAGTCA	AGTTCGAAGG	360
TGACACCCCTG	GTGAATAGAA	TCGAGTTGAA	GGGCATTGAC	400
TTTAAGGAAG	ATGGAAACAT	TCTCGGCCAC	AAGCTGGAAT	440
ACAACATATAA	CTCCCACAAT	GTGTACATCA	TGGCCGACAA	480
GCAAAAGAACAT	GGCATCAAGG	TCAACTTCAA	GATCAGACAC	520
AACATTGAGG	ATGGATCCGT	GCAGCTGGCC	GACCATTATC	560
AACAGAACAC	TCCAATCGGC	GACGGCCCTG	TGCTCCTCCC	600
AGACAACCAT	TACCTGTCCA	CCCAGTCTGC	CCTGTCTAAA	640
GATCCCAACG	AAAAGAGAGA	CCACATGGTC	CTGCTGGAGT	680
TTGTGACCGC	TGCTGGGATC	ACACATGGCA	TGGACGAGCT	720
GTACAAGTGA	GCGCCCTTAAG	GGCCATATGG	TGAGTGGATG	760
CCTTGACCCC	AGGCGGGGAT	GGGGGAGACC	TGTAGTCAGA	800
GCCCCCGGGC	AGCACAGGCC	AATGCCCGTC	CTTCCCTGTC	840
AGGATGAGTA	GTGAGTGCCT	CTCCTGCC	TGGAAGTTGC	880
CACTCCAGTG	CCCACCAAGCC	TTGTCTTAAT	AAAATTAAAGT	920
TGCATCATTT	TGTCTGACTA	GGTGTCTCT	ATAATATTAT	960
AAGCTTGATA	TCGAATTCTT	TCGGACTTTT	GAAAGTGTATG	1000
GTGGTGGGGG	AAGGATTCGA	ACCTTCAAG	TCGATGACGG	1040
CAGATTAGA	GTCTGCTCCC	TTTGGCCGCT	CGGGAACCCC	1080
ACCAACGGTA	ATGCTTTAC	TGGCCTGCTC	CCTTATCGGG	1120
AAGCGGGGCC	CATCATATCA	AATGACGCC	CGCTGTAAAG	1160
TGTTACGTTG	AGAAAAGATT	CCTGCAGCCC	GCCGCGTTGC	1200
TGGCGTTTTT	CCATAGGCTC	CGCCCCCTG	ACGAGCATCA	1240
CAAAAATCGA	CGCTCAAGTC	AGAGGGGGCG	AAACCCGACA	1280
GGACTATAAA	GATACCAGGC	GTTTCCCCCT	GGAAGCTCCC	1320
TCGTGCGCTC	TCCTGTTCCG	ACCCTGCC	TTACCGGATA	1360
CCTGTCCGCC	TTTCTCCCTT	CGGGAAAGCGT	GGCGCTTTCT	1400
CAATGCTCAC	GCTGTAGGTA	TCTCAGTTCG	GTGTAGGTGC	1440
TTCGCTCCAA	GCTGGCTGT	GTGCACGAAC	CCCCCGTTCA	1480
GCCCGACCGC	TGCGCCTTAT	CCGGTAACTA	TCGTCTTGAG	1520
TCCAACCCGG	TAAGACACGA	CTTATCGCCA	CTGGCAGCAG	1560

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CCACTGGTAA	CAGGATTAGC	AGAGCGAGGT	ATGTAGGCCG	1600	
TGCTACAGAG	TTCTTGAAGT	GGTGGCCTAA	CTACGGCTAC	1640	
ACTAGAAGGA	CAGTATTG	TATCTGCGCT	CTGCTGAAGC	1680	
CAGTTACCTT	CGGAAAAAGA	GTTGGTAGCT	CTTGATCCGG	1720	
CAAACAAACC	ACCGCTGGTA	GCGGTGGTTT	TTTTGTTG	1760	
AAGCAGCAGA	TTACCGCGAG	AAAAAAAGGA	TCTGGGGGAT	1800	
CCGGAGAGCT	CCCAACGCGT	TGGATGCATG	GATGAGGGAA	1840	
AGGAGGTAAG	ATCTGTAATG	AATAAGCAGG	AACTTTGAAG	1880	
ACTCAGTGAC	TCAGTGAGTA	ATAAAGACTC	AGTGA	1920	
GATCCTGTCC	TAACTGCCAC	TCCTTGTGT	CCCAAGAAAG	1960	
CGGCTTCCTG	CTCTCTGAGG	AGGACCCCTT	CCCTGGAAGG	2000	
TAAAACTAAG	GATGTCAGCA	GAGAAATTTT	TCCACCATTG	2040	
GTGCTTGGTC	AAAGAGGAAA	CTGATGAGCT	CACTCTAGAT	2080	
GAGAGAGCAG	TGAGGGAGAG	ACAGAGACTC	GAATTCCGG	2120	
AGCTATTTCA	GT	TTTCTTTT	CCGTTTG	CAATTC	2160
TATGATACCG	GCCAATGCTT	GGTTGCTATT	TTGGAAACTC	2200	
CCCTTAGGGG	ATGCCCTCA	ACTGGCCCTA	TAAAGGGCCA	2240	
GCCTGAGCTG	CAGAGGATTC	CTGCAGAGGA	TCAAGACAGC	2280	
ACGTGGACCT	CGCACAGCCT	CTCCCACA		2308	

We claim:

1. A humanized polynucleotide vector comprising:  
a human derived promoter or mammalian homolog thereof which is functional in a target tissue or target cells, said promoter operably linked to a sequence acceptance site which accepts cDNA target products from rtPCR.
2. The humanized polynucleotide vector according to claim 1 wherein the target cells are selected from the group consisting of myocytes and professional antigen presenting cells.
3. The humanized polynucleotide vector according to claim 1 or 2 wherein the target cells or target tissue are human.
4. The humanized polynucleotide vector according to claims 1-3 wherein the human derived promoter is a RANTES promoter or portion thereof.
5. The humanized polynucleotide vector according to claim 4 wherein the promoter has approximately 440 base pairs.
6. The humanized polynucleotide vector according to claims 4 or 5 wherein the portion corresponds to a region spanning the NCO site through the KpnI site of the genomic RANTES promoter
7. The humanized polynucleotide vector according to claims 1-5 or 6 wherein the sequence acceptance site comprises the nucleotide sequence as depicted in Figure 2.
8. The humanized polynucleotide vector according to claims 1-6 or 7 further comprising an origin for replication and growth and a nucleic acid sequence which allows for selection of recombinant plasmids.
9. The humanized polynucleotide vector according to claim 8 wherein the origin for replication is colE1 or functional portion thereof.

10. The humanized polynucleotide vector according to claim 8 wherein the origin for replication comprises a 635 base pair region of the colEl origin of replication.

11. The humanized polynucleotide vector according to claim 1 to 9 or 10 further comprising a human-derived 3' splice sequence and a human-derived poly A sequence, both sequences located downstream of the sequence acceptance site.

12. The humanized polynucleotide vector according to claim 11 wherein the human derived 3' splice and poly A sequence are derived from human growth hormone.

13. A polynucleotide vector according to claims 1-11 or 12 wherein a 5' sequence acceptance site reads on the positive strand as GCCACCATGGCC and a 3' sequence acceptance site reads on the positive strand as GCCTTAAGGGC.

14. A polynucleotide vector comprising SEQ ID No 16, SEQ ID No 27 or SEQ ID No 28.

15. A polynucleotide vector comprising ATCC designation 98400 or ATCC designation 98401.

16. A polynucleotide vector according to claims 1-14 or 15 further comprising a nucleotide sequence encoding at least one target antigen or antigenic epitope thereof alone or in combination with a nucleotide sequence encoding a cytokine or chemokine.

17. A polynucleotide vector vaccine comprising a human derived promoter or mammalian homolog thereof which is functional in a mammalian target tissue or mammalian target cell, said promoter operably linked to a sequence acceptance site and a nucleotide sequence encoding at least one target antigen or antigenic epitope thereof.

18. A polynucleotide vector vaccine according to claim 17 wherein the target antigen is a product of a tumor associated genetic derangement.

19. A polynucleotide vector vaccine according to claim 17 wherein the target antigen is a tumor antigen, bacterial antigen, viral antigen, or parasitic antigen.

20. The polynucleotide vector vaccine according to claims 17 or 18, wherein the tumor antigen is p53, RB, ras, int-2, Hst, Tre17, BRCA-1, BRCA-2, MUC-1, HER-2/neu, truncated EGFRvIII, CEA, MyC, Myb, OB-1, OB-2, BCR/ABL, GIP, GSP, RET, ROS, FIS, SRC, TRC, WT1, DCC, NF1, FAP, MEN-1, ERB-B1 combinations thereof.

21. A polynucleotide vector vaccine according to claim 17, 18, 19 or 20 further comprising a nucleic acid sequence encoding a cytokine or chemokine.

22. A polynucleotide vector vaccine according to claim 21 wherein the cytokine is selected from the group consisting of interleukin 2, interleukin 3, interleukin 4, interleukin 7, interleukin 8, interleukin 12, interleukin 15, GM-CSF, tumor necrosis factor, interferon.

23. A polynucleotide vector vaccine according to claim 21 wherein the chemokine is selected from the group consisting of RANTES, MCP, MIP- $\alpha$ , MIP- $\beta$ , defensins and combinations thereof.

24. A method for expressing at least one target antigen or antigenic epitope thereof in cells comprising:

introducing a humanized polynucleotide vector into said cells, under conditions for expression of the target antigen or antigenic epitope thereof, said vector comprising:

a human derived promoter or mammalian homolog thereof, which is functional in said cells, a sequence acceptance site which accepts cDNA target products from rtPCR cloning and,

a nucleic acid sequence encoding at least one target antigen or antigenic epitope thereof.

25. The method of claim 24 wherein the cells are selected from the group consisting of myocytes and professional antigen presenting cells.

26. The method of claim 24 wherein the target antigen is a tumor antigen bacterial antigen, viral antigen, or parasitic antigen.

27. The method of claim 26 wherein the tumor antigen is p53, RB, ras, int-2, Hst, Tre 17, BRCA-1, BRCA-2, MUC-1, HER-2/neu, truncated EGFRvIII, CEA, MyC, Myb, OB-1, OB-2, BCR/ABL, GIP, GSP, RET, ROS, FIS, SRC, TRC, WT1, DCC, NF1, FAB, MEN-1, ERB-B1 or combinations thereof.

28. A pharmaceutical composition comprising at least one polynucleotide vector according to claims 1-15 or 16 and a pharmaceutically acceptable carrier.

29. A pharmaceutical composition comprising the polynucleotide vector vaccine according to claims 17-22 or 23 and a pharmaceutically acceptable carrier.

30. A kit comprising the polynucleotide vector according to claims 1-15 or 16.

31. A kit according to claim 30 further comprising a nucleotide sequence encoding at least one target antigen or antigenic epitope thereof.

32. A kit comprising the polynucleotide vector vaccine according to claims 17-22 or 23.

33. A kit according to claim 32, further comprising an expression enhancing agent.

34. The kit according to claim 33 wherein the expression enhancing agent

is a mycotoxic agent.

35. The kit according to claim 34 wherein the mycotoxic agent is bupivacaine-HCl and dextrose.

36. A host cell comprising:  
the polynucleotide vector of claim 17-22 or 23, wherein the host cell is capable of expressing the target antigen or antigenic epitope.

37. The host cell according to claim 36 wherein the host cell is a myocyte or professional antigen presenting cell.

38. A method of stimulating a specific immune response to at least one target antigen or antigenic epitope thereof in a mammal comprising: administration of an effective amount of polynucleotide vector vaccine according to claim 17-22 or 23 into the mammal, said amount elicits the specific immune response to the target antigen or epitope thereof.

39. The method according to claim 38, wherein a site of administration is muscle or skin.

40. The method according to claim 38 further comprising administration of effective amount of an expression enhancing agent prior to administration of the polynucleotide vector vaccine.

41. The method according to claim 40 wherein the expression enhancing agent is a mycotoxic agent.

42. The method according to claim 41 wherein the mycotoxic agent is bupivacaine-HCl or dextrose.

43. The method according to claim 38-41 or 42 wherein the target antigen is a tumor antigen, bacterial antigen, viral antigen or parasitic antigen.

44. The method according to claim 43 wherein the tumor antigen is selected from the group consisting of P53, RB, ras, int-2, Hst, Tre 17, BRCA-1, BRCA-2, MUC-1, HER-2/neu, truncated EGFRvIII, CEA, MyC, Myb, OB-1, OB-2, BCR/ABL, GIP, GSP, RET, ROS, FIS, SRC, TRC, WT1, DCC, NFI, FAB, MEN-1, ERB-B1 and combinations thereof.

45. The method according to claim 44 wherein the method generates antigen specific cytotoxic lymphocytes to the tumor antigen or antigenic epitopes thereof.

46. A method of making a humanized polynucleotide vector comprising:  
operably linking a human derived promoter or mammalian homolog thereof which is functional in a target tissue or target cells to a sequence acceptance site, said site accepts cDNA target products from rtPCR.

47. The method according to claim 46, wherein the human derived promoter is a RANTES promoter or portion thereof.

48. A isolate antibody comprising an antibody elicited in response to immunization with the polynucleotide vector vaccine according to claim 17-22 or 23, said antibody is specific for the target antigen or antigenic epitope thereof.

49. A sequence acceptance site comprising nucleic acid sequences which accept cDNA target products from rtPCR cloning.

50. The sequence acceptance site according to claim 49 wherein the sequence acceptance site directionally accepts target sequence specific products from rtPCR cloning via unique sites within an interrupted palindrome recognition sequence for a restriction endonuclease.

51. The sequence acceptance site according to claim 50 wherein the restriction endonuclease is Bgl I.

52. The sequence acceptance site according to claim 49, 50 or 51 wherein

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a 5' acceptance site reads on the positive strand as GCCACCATGGCC.

53. The sequence acceptance site according to claim 52 wherein a 3' acceptance site reads on the positive strand as GCCTTAAGGGC.

54. The sequence acceptance site according to claim 49 wherein the site comprises the nucleotide sequence as depicted in Figure 2.

**AMENDED CLAIMS**

[received by the International Bureau on 22 January 1998 (22.01.98);  
original claims 1-54 replaced by new claims 1-66 (8 pages)]

**1. A humanized polynucleotide vector comprising:**

a human derived promoter or mammalian homolog thereof which is functional in a target tissue or target cells, said promoter operably linked to a sequence acceptance site which directionally accepts cDNA target products from rtPCR cloning via unique sites within an interrupted palindrome recognition sequence for a restriction endonuclease, said vector lacking nucleic acid sequences encoding vector-derived polypeptides.

**2.** The humanized polynucleotide vector according to claim 1 wherein the target cells are selected from the group consisting of myocytes and professional antigen presenting cells.

**3.** The humanized polynucleotide vector according to claim 1 or 2 wherein the target cells or target tissue are human.

**4.** The humanized polynucleotide vector according to claims 1-3 wherein the human derived promoter is a RANTES promoter or portion thereof.

**5.** The humanized polynucleotide vector according to claim 4 wherein the promoter has approximately 440 base pairs.

**6.** The humanized polynucleotide vector according to claims 4 or 5 wherein the portion corresponds to a region spanning the NCO site through the KpnI site of the genomic RANTES promoter

**7.** The humanized polynucleotide vector according to claims 1-5 or 6 wherein the sequence acceptance site comprises the nucleotide sequence as depicted in Figure 2.

**8.** The humanized polynucleotide vector according to claims 1-6 or 7 further comprising an origin for replication and growth and a nucleic acid sequence which allows for selection of recombinant plasmids.

**9.** The humanized polynucleotide vector according to claim 8 wherein the origin for replication is coIE1 or functional portion thereof.

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10. The humanized polynucleotide vector according to claim 8 wherein the origin for replication comprises a 635 base pair region of the colE1 origin of replication.

11. The humanized polynucleotide vector according to claim 1 to 9 or 10 further comprising a human-derived 3' splice sequence and a human-derived poly A sequence, both sequences located downstream of the sequence acceptance site.

12. The humanized polynucleotide vector according to claim 11 wherein the human derived 3' splice and poly A sequence are derived from human growth hormone.

13. A polynucleotide vector according to claims 1-11 or 12 wherein a 5' sequence acceptance site reads on the positive strand as GCCACCATGGCC and a 3' sequence acceptance site reads on the positive strand as GCCTTAAGGGC.

14. A polynucleotide vector comprising SEQ ID No 16, SEQ ID No 27 or SEQ ID No 28.

15. A polynucleotide vector comprising ATCC designation 98400 or ATCC designation 98401.

16. A polynucleotide vector according to claims 1-14 or 15 further comprising a nucleotide sequence encoding at least one target antigen or antigenic epitope thereof alone or in combination with a nucleotide sequence encoding a cytokine or chemokine.

17. A polynucleotide vector vaccine comprising a human derived promoter or mammalian homolog thereof which is functional in a mammalian target tissue or mammalian target cell, said promoter operably linked to a sequence acceptance site which directionally accepts cDNA target products from rtPCR cloning via unique sites within an interrupted palindrome recognition sequence for a restriction endonuclease, a nucleotide sequence encoding at least one target antigen or antigenic epitope thereof and lacking nucleic acid sequences encoding vector-derived polypeptides.

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18. A polynucleotide vector vaccine according to claim 17 wherein the target antigen is a product of a tumor associated genetic derangement.

19. A polynucleotide vector vaccine according to claim 17 wherein the target antigen is a tumor antigen, bacterial antigen, viral antigen, or parasitic antigen.

20. The polynucleotide vector vaccine according to claims 17 or 18, wherein the tumor antigen is p53, RB, ras, int-2, Hst, Tre17, BRCA-1, BRCA-2, MUC-1, HER-2/neu, truncated EGFRvIII, CEA, MyC, Myb, OB-1, OB-2, BCR/ABL, GIP, GSP, RET, ROS, FIS, SRC, TRC, WT1, DCC, NF1, FAP, MEN-1, ERB-B1 or combinations thereof.

21. A polynucleotide vector vaccine according to claim 17, 18, 19 or 20 further comprising a nucleic acid sequence encoding a cytokine or chemokine.

22. A polynucleotide vector vaccine according to claim 21 wherein the cytokine is selected from the group consisting of interleukin 2, interleukin 3, interleukin 4, interleukin 7, interleukin 8, interleukin 12, interleukin 15, GM-CSF, tumor necrosis factor, and interferon.

23. A polynucleotide vector vaccine according to claim 21 wherein the chemokine is selected from the group consisting of RANTES, MCP, MIP-1 $\alpha$ , MIP-1 $\beta$ , defensins, IP-10 and combinations thereof.

24. A method for expressing at least one target antigen or antigenic epitope thereof in cells comprising:

introducing a humanized polynucleotide vector into said cells, under conditions for expression of the target antigen or antigenic epitope thereof, said vector comprising:

a human derived promoter or mammalian homolog thereof, which is functional in said cells, a sequence acceptance site which directionally accepts cDNA target products from rtPCR cloning via unique sites within an interrupted palindrome recognition sequence for a restriction endonuclease and,

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a nucleic acid sequence encoding at least one target antigen or antigenic epitope thereof, and lacking nucleic acid sequences encoding vector-derived polypeptides.

25. The method of claim 24 wherein the cells are selected from the group consisting of myocytes and professional antigen presenting cells.

26. The method of claim 24 wherein the target antigen is a tumor antigen bacterial antigen, viral antigen, or parasitic antigen.

27. The method of claim 26 wherein the tumor antigen is p53, RB, ras, int-2, Hst, Tre 17, BRCA-1, BRCA-2, MUC-1, HER-2/neu, truncated EGFRvIII, CEA, MyC, Myb, OB-1, OB-2, BCR/ABL, GIP, GSP, RET, ROS, FIS, SRC, TRC, WT1, DCC, NF1, FAB, MEN-1, ERB-B1 or combinations thereof.

28. A pharmaceutical composition comprising at least one polynucleotide vector according to claims 1-15 or 16 and a pharmaceutically acceptable carrier.

29. A pharmaceutical composition comprising the polynucleotide vector vaccine according to claims 17-22 or 23 and a pharmaceutically acceptable carrier.

30. A kit comprising the polynucleotide vector according to claims 1-15 or 16. "

31. A kit according to claim 30 further comprising a nucleotide sequence encoding at least one target antigen or antigenic epitope thereof.

32. A kit comprising the polynucleotide vector vaccine according to claims 17-22 or 23.

33. A kit according to claim 32, further comprising an expression enhancing agent.

34. The kit according to claim 33 wherein the expression enhancing agent

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35. The method according to claim 43 wherein the tumor antigen is selected from the group consisting of P53, RB, ras, int-2, Hst, Tre 17, BRCA-1, BRCA-2, MUC-1, HER-2/neu, truncated EGFRvIII, CEA, MyC, Myb, OB-1, OB-2, BCR/ABL, GIP, GSP, RET, ROS, FIS, SRC, TRC, WT1, DCC, NF1, FAB, MEN-1, ERB-B1 and combinations thereof.

36. The method according to claim 44 wherein the method generates antigen specific cytotoxic lymphocytes to the tumor antigen or antigenic epitopes thereof.

37. A method of making a humanized polynucleotide vector comprising: operably linking a human derived promoter or mammalian homolog thereof which is functional in a target tissue or target cells to a sequence acceptance site, said site directionally accepts cDNA target products from rtPCR cloning via unique sites within an interrupted palindrome recognition sequence for a restriction endonuclease, said vector lacking nucleic acid sequences encoding vector-derived polypeptides.

38. The method according to claim 46, wherein the human derived promoter is a RANTES promoter or portion thereof.

39... A isolate antibody comprising an antibody elicited in response to immunization with the polynucleotide vector vaccine according to claim 17-22 or 23, said antibody is specific for the target antigen or antigenic epitope thereof.

50. The sequence acceptance site comprising nucleic acid sequences which accept cDNA target products from rtPCR cloning wherein the sequence acceptance site directionally accepts target sequence specific products from rtPCR cloning via unique sites within an interrupted palindrome recognition sequence for a restriction endonuclease.

51. The sequence acceptance site according to claim 50 wherein the restriction endonuclease is Bgl I.

52. The sequence acceptance site according to claim 50 or 51 wherein

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a 5' acceptance site reads on the positive strand as GCCACCATGGCC.

53. The sequence acceptance site according to claim 52 wherein a 3' acceptance site reads on the positive strand as GCCTTAAGGGC.

54. The sequence acceptance site according to claim 50 wherein the site comprises the nucleotide sequence as depicted in Figure 2.

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55. A use of a polynucleotide vector vaccine in the manufacture of a medicament for use in a method of stimulating a specific immune response to at least one target antigen or antigenic epitope thereof in a mammal, said method comprising:

administration of an effective amount of a polynucleotide vector vaccine according to claims 17-22 or 24 into the mammal, said amount elicits the specific immune response to the target antigen or epitope thereof.

56. A use according to claim 55, wherein a site of administration is muscle or skin.

57. A use according to claim 55 or 56 further comprising the use of an expression enhancing agent in the manufacture of a medicament for use in the method, the method further comprising administration of an effective amount of an expression enhancing agent prior to administration of the polynucleotide vector vaccine.

58. The use according to claim 57, wherein the expression enhancing agent is a mycotoxic agent.

59. The use according to claim 58, wherein the mycotoxic agent is bupivacaine-HCl or dextrose.

60. The use according to claims 55-58 or 59 wherein the target antigen is a tumor antigen, bacterial antigen, viral antigen or parasitic antigen.

61. The use according to claim 60, wherein the tumor antigen is selected from the group consisting of p53, RB, ras, int-2, Hst, Tre 17, BRCA-1, BRCA-2, MUC-1, HER-2/neu, truncated EGFRvIII, CEA, MyC, Myb, OB-1, OB-2, BCR/ABL, GIP, GSP, RET, ROS, FIS, SRC, TRC, WT1, DCC, NF1, FAB, MEN-1, ERB-B1 and combinations thereof.

62. The use according to claim 61, wherein the method generates antigen specific cytotoxic lymphocytes to the tumor antigen or antigenic epitopes thereof.

63. The humanized polynucleotide vector according to claims 1-15 or 16, wherein the vector lacks an antibiotic resistance encoding nucleic acid sequence.

64. The humanized polynucleotide vector according to claims 1-15 or 16, wherein the recognition sequence is recognized by Bgl I restriction endonuclease.

65. The humanized polynucleotide vector according to claim 8, wherein the

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nucleic acid sequence which allows for selection is a suppressor tRNA gene, a synthetic SupF complementation tRNA gene, or functional derivatives thereof.

66. The humanized polynucleotide vector according to claim 65, wherein the nucleic acid sequence is selected from the group consisting of SupE, SupP, SupD, SupU, SupF, SupZ, glyT, glyU, SerP, psu<sub>2</sub><sup>+</sup>, psu<sub>2</sub><sup>+</sup>-C34, psu<sub>2</sub><sup>+</sup>AM and psu<sub>2</sub><sup>+</sup>OC.

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STATEMENT UNDER ARTICLE 19

In response to the Notification of Transmittal of the International Search Report or Declaration dated November 27, 1997, Applicants are forwarding herein replacement sheets of amended claims.

Claims 1, 17, 24 and 46 were amended to describe the sequence acceptance site as recited in claim 50. Claims 1, 17, 24 and 46 were further amended to recite "lacking nucleic acid sequences encoding vector-derived polypeptides."

Claims 20 and 22 were amended to correct grammatical errors.

Claim 23 was amended to correct a typographical error in MIP-1 $\alpha$  and to insert the chemokine "IP-10".

The recitation from cancelled claim 49 was incorporated into claim 50.

Claim 52 was amended to reflect the cancellation of claim 49.

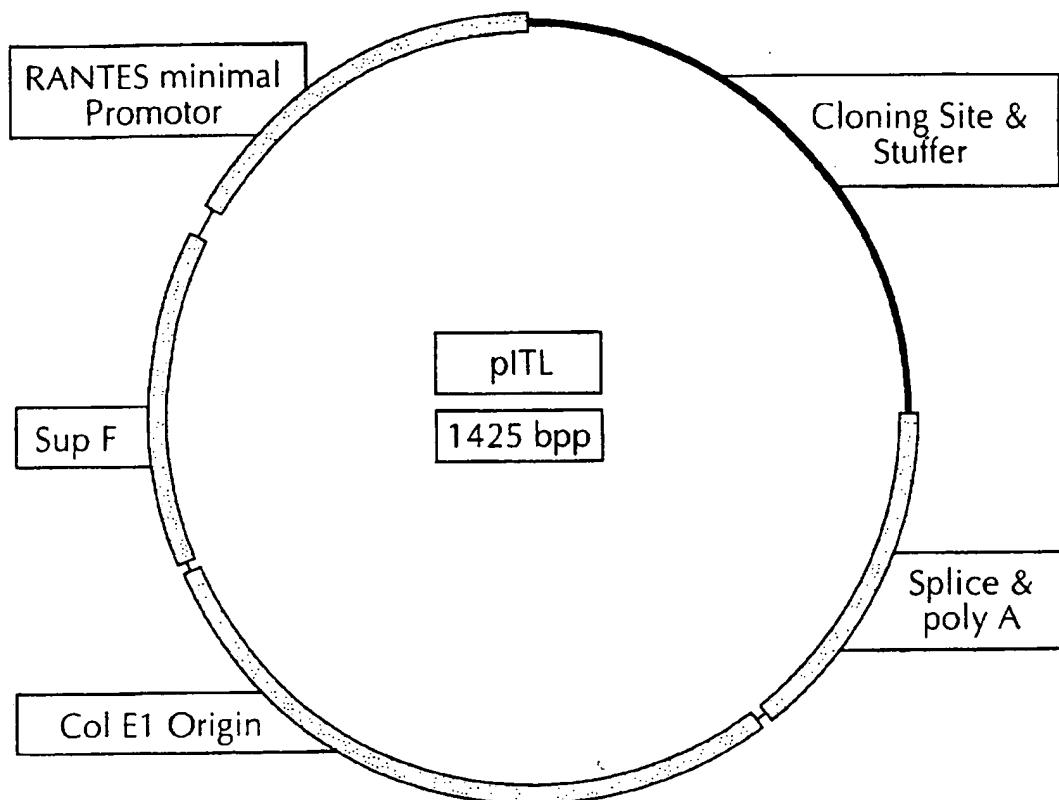
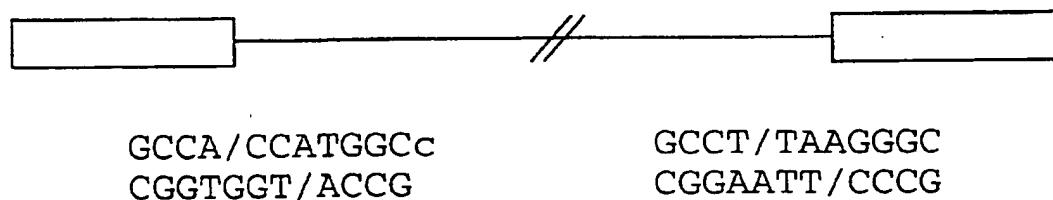
Claim 54 was amended to depend from claim 50.

New "use of product" claims were added, i.e. claims 55-62 which mirror the method of treatment claims, claims 38-45.

New claims 63-66 were added which further characterize the vector.

Applicants respectfully submit that the amendment does not introduce new matter and are fully supported by, and do not change the meaning or content of the description and drawings.

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**FIG. 1****FIG. 2****SUBSTITUTE SHEET (RULE 26)**

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FIG. 3A

FIG. 3B

**FIG. 3C**

929

THQSDVWSYGVTVWELMTFGSKPYDGIPASEISSILEKGERLPQQPICTIDVYIMMVKCWMIDADSRPKFRELII  
 THQSDVWSYGVTVWELMTFGAKPYDGIPAREIPDLEKGERLPQQPICTIDVYIMMVKCWMIDSECCRPRFRELVS  
 940

EF SKMARDPQRYLVIQGDERMHLPSPTDSNFYRALMDEE-DMDDVVDADEYLIPQQGFF-----  
 EF SRMARDPQRFVVIQN-EDLGPA SP LDSTFYRSL-LEDDDMGDLVDAEYLV PQQGFFCPDPAPGAGGMVHHRH  
 1000

-----SSPSTSRTPLSSLSATSSNNSTVACIDRN--GLQSCP IKEDSF LQRYSSDPTTGAL-T  
 RSSSTRSGGGDLTIGLEPSEEAPRSPLAPSEGAGSDVFDGDLGMGA AKGLQLSPTHDPSPLQRYSEDPTVPLPS  
 1137

E-D--SIDDTFLPVPEYINQ-SVPKRPAGSVQNPVYHNQPLN PAPS RDPHYQD PH STAVGNPEYLN TVQPTCVNS  
 ET DGYVAPLTCSPQPEYVNQPDVRPQPPSPREGPLPAARPA GATLERPKTLSPGKNGVVKDVFAFGGAVENPEYLN  
 1120

TF DSTFLPVPEYINQ-SVPKRPAGSVQNPVYHNQPLN PAPS RDPHYQD PH STAVGNPEYLN TVQPTCVNS TFDSP  
 TPQGTCS P QPEYVNQPDVRPQPPSPREGPLPAARPA GATLERPKTLSPGKNGVVKDVFAFGGAVENPEYLT P QGG  
 1300

AHWAQKGSHQ- ISL--DNPDYQ-QDFFPKEAKPNGLFKGST--AENA EYLR-VAPOSSEFIGA  
 AAP-QPHPPPAFSPA FDNLYYWDQDPERGAPPS-TFKG-TPTAENPEYLG LDVPV  
 1255

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FIG. 4A

57  
MELAACRWGLLALLPPGAASTQVCTGTDMLRKRLPASPETHLDMLRHLYQGCQVVQGNLELTYLPTNASLS  
MIIMELAAWCRWGFLLLALLPPGIAGTQVCTGTDMLRKRLPASPETHLDMLRHLYQGCQVVQGNLELTYVPANASLS  
60

296

GGC	ARG	KGP	L	P	TD	C	HE	Q	CAAG	GCT	GPK	KHS	DCL	A	CL	H	F	N	HSG	ICEL	HCP	ALV	TYNT	DTF	ESMPN	PEGRY	TFGASC		
SGC	AR	C	KGR	L	P	T	D	C	HE	Q	CAAG	GCT	GPK	KHS	DCL	A	CL	H	F	N	HSG	ICEL	HCP	ALV	TYNT	DTF	ESMHN	PEGRY	TFGASC

300

TACPYNYLSTDVGSCTLVCPPLHNOEVTAEDGTQRCERKCSKP  
CARVCYGLGMELREVRRAVTSANIQEFAGCKKIF  
TTCPYNYLSTEVGSCTLVCPNNQEVTAEDGTQRCERKCSKP  
CARVCYGLGMELRGARAIITSDNVQEFDGCKKIF  
356  
360

GSLAFLPESFDGDPASNTAPLOPEQLQVFETLEEITGYLYISAWMPDSLQVIRGRIHLHNGAYSLTLQ  
GSLAFLPESFDGDPSSCGIAPLRLPEQLQVFETLEEITGYLYISAWPDSLRLSVFQNLRIIRGRIHLHDGAYSLTLQ

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**FIG. 4B**

GLG ISWLGRLS LRELGSGGL ALIHHNTHL CFVHTV PWDQ LFRN PHQ ALL HTAN RPEDE - C VGEGL ACHOLCARGHC  
 GLGIHSLGLRSL RELGSGGL ALIHRNAHIL CFVHTV PWDQ LFRN PHQ ALL HSGNRPEE D LCVSSGLVCNSLCAHGH  
 476

WGP GPT QCVNC SQFLRG QEC VEE C RVL QGL PREY VNAR HCL PCHPEC QP QONG SVT CFG PEA DQC VACA HYKD PPF  
 WGP GPT QCVNC SHFLRG QEC VEE C RVL QGL PREY VSDK RCL PCHPEC QP QNS SET CFG SEADQC ACA HYKD SSS  
 535

CVARCP SGVK PDL SYMP IWK FP DEEG ACQ PCP IN CT HSC VD LDK GC PAE Q RASPL TSI I SAVV GILL VV LGW  
 CVARCP SGVK PDL SYMP IWK YP DEEG IC QPC P IN CT HSC VD LDER GC PAE Q RASPV TFI ATVEGV LLFL ILV VY  
 540

EGILL KRR - QKIR KYTM RRL QET E LVEPL TPSG AMPN QAM RIL KET E LRK V KV LGSGA FGT VYKG I WI PD  
 :  
 VGILL KRR RQ - KIR KYTM RRL QET E LVEPL TPSG AMPN QAM RIL KET E LRK V KV LGSGA FGT VYKG I WI PD  
 595

GENVK I PVA I KV LRENTSPK ANKE I LDEAY V MAGVG SP YY SRL LGIC LT STV QLV TQL MPY GCL LDHV REN RGRL  
 :  
 GENVK I PVA I KV LRENTSPK ANKE I LDEAY V MAGVG SP YY SRL LGIC LT STV QLV TQL MPY GCL LDHV REH RGRL  
 660

655

EGILL KRR - QKIR KYTM RRL QET E LVEPL TPSG AMPN QAM RIL KET E LRK V KV LGSGA FGT VYKG I WI PD  
 :  
 VGILL KRR RQ - KIR KYTM RRL QET E LVEPL TPSG AMPN QAM RIL KET E LRK V KV LGSGA FGT VYKG I WI PD  
 710

GENVK I PVA I KV LRENTSPK ANKE I LDEAY V MAGVG SP YY SRL LGIC LT STV QLV TQL MPY GCL LDHV REN RGRL  
 :  
 GENVK I PVA I KV LRENTSPK ANKE I LDEAY V MAGVG SP YY SRL LGIC LT STV QLV TQL MPY GCL LDHV REH RGRL  
 775

GSQ DLL NWCM QIA KGM S YLED VRL V HRDL AARN VL VKSP NHVK IT DFG L ARLL DIDE TEY HADGG KV PI KW MALE  
 GSQ DLL NWCM VQIA KGM S YLED VRL V HRDL AARN VL VKSP NHVK IT DFG L ARLL DIDE TEY HADGG KV PI KW MALE  
 835

870

896

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FIG. 4C

SILRRRFTHQSDVWSYGVTVWELMTFGAKPYDGIPIAREIPDLLEKGERLPPPICTIDVYMMIMVKCWMIDSEC  
 RP  
 SILRRRFTHQSDVWSYGVTVWEMTFGAKPYDGIPIAREIPDLLEKGERLPPPICTIDVYMMIMVKCWMIDSEC  
 RP  
 955

RFRELVSEFSRMARDPQRFVVIQNEIDLGPASPLDSTFYRSLLEDGGDLDVDAEELYLPQQGFFCPDPAPGAGGM  
 :  
 RFRELVSEFSRMARDPQRFVVIQNEIDLGPSSPMMDSTFYRSLLEDGGDLDVDAEELYLPQQGFFSPDPTPGTG  
 ST

1010

VHHHRHRSSTRSGGDLTLGLEPSEEAPRSPLAPSEGAGSDVFDGDLGMGAAKGLQSLPTHDPSPSPLQRYSEDPT  
 :  
 AHRRHRSSTRSGGELTLGLEPSEEGBPRSRPLAPSEGAGSDVFDGDLAMGVTKGLQSLSPHDLSPLQRYSEDPT

1015

1070

VPLPSETDGYVAPLTCSPQPEYVNQPDVRPQQPSREGPLPAARPAGATLERPKTLSPGKNGVVKDVFAGGAVE  
 :  
 LPLPPETDGYVAPLACSPQPEYVNQSEVQPQQPLTPEGPLPPVVRPAGATLERPKTLSPGKNGVVKDVFAGGAVE

1075

1130

NPEYLTPOGGAAPOPHPPPAFSPAEDNLYYMDQDPPERCAPPSTFKGTPTAENPEYLGDDVPY  
 :  
 NPEYLVPREGTASPPHPSPAFSPAFDNLYYWDQNSSEQGCPPPSNFEGTPTAENPEYLGDDVPY

1135

1193

NPEYLTPOGGAAPOPHPPPAFSPAEDNLYYMDQDPPERCAPPSTFKGTPTAENPEYLGDDVPY  
 :  
 NPEYLVPREGTASPPHPSPAFSPAFDNLYYWDQNSSEQGCPPPSNFEGTPTAENPEYLGDDVPY

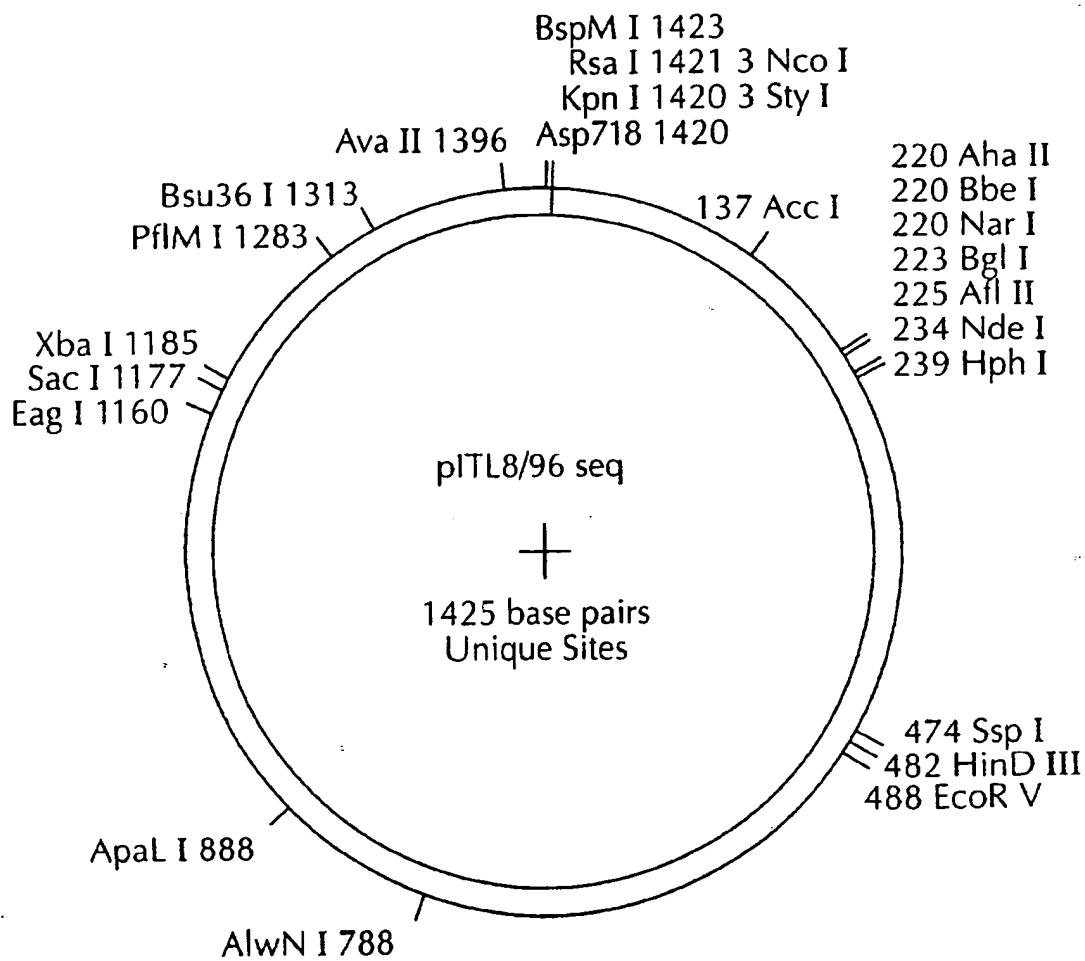
1198

1255

NPEYLTPOGGAAPOPHPPPAFSPAEDNLYYMDQDPPERCAPPSTFKGTPTAENPEYLGDDVPY  
 :  
 NPEYLVPREGTASPPHPSPAFSPAFDNLYYWDQNSSEQGCPPPSNFEGTPTAENPEYLGDDVPY

1263

**SUBSTITUTE SHEET (RULE 26)**

**FIG. 5**

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/14306

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/85 A61K48/00 C12N5/10 C07K16/32 C07K16/30  
 C12Q1/68 C12N15/11

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N A61K C07K C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 07347 A (BIO RAD LABORATORIES) 16 March 1995	49
Y	see page 2, line 36 - page 4, line 37 see page 7, line 8 - line 32 see page 8, line 31 - page 9, line 14 see page 10, line 9 - line 35 see page 12, line 12 - page 14, line 2; example 2 --- -/-	1-5, 17, 24, 25, 36, 37

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

## \* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"Z" document member of the same patent family

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Date of the actual completion of the international search

17 November 1997

Date of mailing of the international search report

27.11.97

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Montero Lopez, B

## INTERNATIONAL SEARCH REPORT

Inter. nat Application No

PCT/US 97/14306

(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	COLOMA, M. JOSEFINA ET AL: "Novel vectors for the expression of antibody molecules using variable regions generated by polymerase chain reaction" J. IMMUNOL. METHODS (1992), 152(1), 89-104 CODEN: JIMMBG; ISSN: 0022-1759, 1992, XP000289684	49
Y	see abstract see page 90, right-hand column, paragraph 2 see page 92, right-hand column, paragraph 3 - right-hand column, paragraph 3 ---	1-5
X	WO 92 01055 A (BOEHRINGER INGELHEIM INT) 23 January 1992 see page 4, paragraph 2 - page 5, paragraph 1; examples 1-5 ---	49
Y	PETER J. NELSON ET AL.: "Genomic organization and transcriptional regulation of the RANTES chemokine gene" JOURNAL OF IMMUNOLOGY, vol. 151, no. 5, 1 September 1993, BALTIMORE US, pages 2601-2612, XP002047102 cited in the application see abstract see page 2601, right-hand column, paragraph 2 - page 2602, left-hand column, paragraph 1 see page 2603, right-hand column, paragraph 2 - page 2604, left-hand column, paragraph 1 see page 2608, left-hand column, paragraph 2 - page 2610, left-hand column, paragraph 1 -----	1-5,17, 24,25, 36,37

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 97/14306

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:  
see FURTHER INFORMATION sheet PCT/ISA/210
2.  Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Remark : Although claims 38-45 are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

## INTERNATIONAL SEARCH REPORT

Information on patent family members

Interr. Application No.

PCT/US 97/14306

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9507347 A	16-03-95	US 5426039 A CA 2171096 A EP 0722487 A JP 9502350 T	20-06-95 16-03-95 24-07-96 11-03-97
WO 9201055 A	23-01-92	DE 4021917 A DE 4035877 A AU 650893 B AU 8208291 A DE 59101397 D EP 0538300 A ES 2063515 T HU 65846 A JP 6502987 T SK 386392 A	16-01-92 14-05-92 07-07-94 04-02-92 19-05-94 28-04-93 01-01-95 28-07-94 07-04-94 10-08-94